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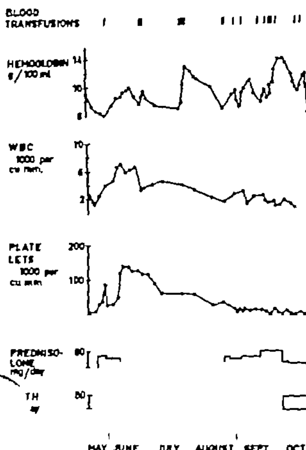


Fig 1 Course of the disease and treatment.

In the beginning of July the severe back pains returned, and the patient had also pains localized to the extremities and the chest. The pain had an agonizing, boring character and the bones were very tender on palpation. During the next t months the pain had an intermittent course, and it was observed that the most severe attacks were accompanied by fever and a prompt fall in the blood cell counts. Prednisolone in increased dosage and ACTH had little or no effect. From the beginning of September the patient had more or less continuous pains and the pancytopenia became extreme reaching minimum values of WBC 440/mm³ and platelets 2,000/mm³. Blood and platelet transfusions gave an increase in hemoglobin concentration but influenced the counts of leucocytes and platelets very little. There was no gross hemorrhage but skin purpura and petechial bleedings from mucous membranes. Peripheral lymphadenopathy or splenomegaly did not appear. Repeated X-ray examinations of the skeleton revealed no changes except for her earlier degenerative spondylitis. Her general condition grew worse, she developed candida stomatitis and esophagitis, and she died on October 20, 1968.

Autopsy findings

The mucous membranes of the tongue and esophagus ere covered by black crusts. The liver was enlarged (2,000 g) with smooth dark-brown surface. No gross infiltrates were seen on cut surfaces. The gall-bladder was fibrous and firmly contracted around a concrement. The spleen was somewhat enlarged (250 g), without gross infiltrates. Several slightly enlarged lymph nodes, up to the size of a hazel-nut, were found para-aortally in the abdomen. No enlargement of lymph nodes was found elsewhere. The red bone-marrow in the thoracic and lumbar spine showed extensive, interspersed, yellow-colored areas. In the iliac crests and sternum the marrow was grossly normal. Red bone marrow was also found in the femur shafts. The remaining organs were unremarkable.

On microscopic examination, the bone marrow in lumbar and thoracic spine, ribs, sternum and iliac crests showed the same kind of changes. The marrow was highly cellular and practically devoid of fat. It was dominated by cells with dense, hyperchromatic nuclei and scanty cytoplasm. The smaller of these cells looked like lymphocytes, but most cells were larger, with the appearance of immature lymphocytes or lymphoblasts. A small number of apparently normal myeloic and erythropoietic cells were present. There was a diffuse, marked increase in reticulin, but no diffuse fibroblast proliferation. The most cellular parts contained large necroses involving marrow and medullar bone. The necroses were partly structureless with small hemorrhages. Peripherally few minor scar-like areas were found with fibroblast proliferation and collagen deposition. Adjacent to necroses but also in the periosteum several thrombotized vessels were found, some of them infiltrated with lymphoid cells. Thrombi in organization were also seen. Bone marrow changes as described above were found in the femur shafts, but there were no necroses.

All examined abdominal lymph nodes exhibited the picture of malignant lymphadenosis, with diffuse, dense infiltration of lymphoid cells, obliterating follicles and sinuses. There was a moderate cellular polymorphism, but no giant cells. The lymphoid cells infiltrated the lymph node capsules and surrounding adipose tissue. The spleen was densely infiltrated by largely immature lymphoid cells. In the liver large infiltrates of lymphoid cells were found in periportal areas. In addition, there was pronounced vascular congestion in the spleen and in the liver

In the kidneys there was a moderately advanced nephrosclerosis The lungs showed signs of vascular congestion, but no cellular infiltrates. In the esophagus a non-specific, necrotizing inflammation was seen in the mucous membrane.

On the basis f the cellular morphology of the infiltrates in bone marrow lymph nodes, spleen and liver, the diagnosis of lymphatic leukemia was erified.

COMMENTS

The present case, exhibiting a rapid clinical course and an immature lymphatic cell morphology should best be classified as an acute or subacute lymphocytic leukemia.

Acute lymphocytic leukemia is infrequently met with in older ages (1 2) Splenomegaly and lymphadenopathy usually are less conspicuous in acute lymphocytic leukemia than in the chronic form of the disease. These findings may even be absent, both on palpation and X-ray examination as in the case reported. Severe pancytopenia was a dominating feature throughout the course of illness, and was very little influenced by prednisolone therapy On the whole the differential counts of leukocytes were normal except for a few myelocytes, metamyelocytes and nucleated red cells. It has been suggested (4) that low peripheral white blood counts in cases of acute leukemia might be attributed to infarction of large portions of bone marrow with little remaining viable marrow capable of releasing white cells into the peripheral blood. Kundel et al. (3) observed that bone marrow infarction in acute lymphocytic leukemia was followed by a period of pancytopenia. In the present case we repeatedly observed a prompt fall in hemoglobin concentration, white blood cell and platelet counts after severe attacks of bone pain.

The major complaint of the present patient was extremely severe skeletal pain localized to the extremities, large joints, back and sternum. The pains preceded suspicion of a blood dyscrasia by about one month. Objective joint changes never appeared. Apart from periodically intermittent and periodically continuous agonizing, boring ache the bones were very tender on palpation.

The bone marrow during life and at autopsy showed a definite increase in marrow reticulin but no fibroblastic proliferation as seen in primary myelofibrosis. Although the marrow was extremely cellular repeated sternal marrow aspirations were unsuccessful, obviously due to the augmentation of marrow reticulin (3). Increase in bone marrow reticulin is a frequent finding in acute lymphocytic leukemia, especially when affecting the adult (3) Concomitantly with marked increase in marrow reticulin, Kundel et al. (3) observed bone infarction and pain, and they concluded that infarction is one of the causes of bone pain in acute lymphocytic leukemia and the major cause of severe bone pain. In the present case no necrosis were found during life in the marrow specimens examined. At autopsy however very widespread infarctions were observed. These had not produced any specific radiographic changes, in accordance with the experience of Kundel et al. (3) However radiographic changes have been described by others (5 6). The infarctions occurred in the most cellular parts of the bone marrow Observed thrombi in the marrow and periosteum may be a major cause of the infarctions. In contrast to acute lymphocytic leukemia, bone marrow infarction is rarely seen in acute myelogenous leukemia (3 4).

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URINARY EXCRETION OF ADRENAL HORMONES IN MAN

Effects of Ethanol Ingestion, and their Modification by Chlormethiazole

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Abstract A large single dose of ethanol (approximately 900 ml brand whisky) was administered to nine young, healthy males. This stimulus provoked pronounced increases in adrenaline and noradrenaline excretion during and soon after the ethanol ingestion. Similarly the hang-over next morning was accompanied by marked increases in adrenaline and noradrenaline excretion levels, in addition to increased excretion rates of 17-hydroxycorticosteroids. In five subjects treated with 0.5 g chlormethiazole, the catecholamine increases during the hang-over period were significantly reduced. During the week following the ethanol ingestion, the increase in adrenal function tended to persist. Some theoretical and clinical implications of these findings are discussed and some indications in favour of a relationship between the emotional–behavioural and the physiological effects of ethanol ingestion are mentioned.

The studies reported here formed part of a pilot investigation into the effects of large doses of ethanol, alone or combined with chlormethiazole, on a number of liver enzymes in serum. The main results of the enzyme study have already been published (8). The investigation also included measurements of sympathoadrenomedullary and adrenocortical activities during and after ethanol intoxication and their modification by chlormethiazole. The present report is confined to these endocrine and pharmacological aspects.

The evidence presented so far indicates that the ingestion of moderate or large single doses of ethanol is followed by an immediate activation of the adrenal cortex and medulla, in animals as well as in man, as reflected in an increase in the levels of the corresponding hormones in urine (1 4 13, 34–36 38 41 43).

Administering large doses of bourbon whisky to four male alcoholics every four hours, day and night, for up to 27 consecutive days, Mendelson et al. (31) found a marked elevation of serum cortisol levels while the subjects were drinking. Following cessation of drinking, serum cortisol tended to return to pre-drinking levels, although some elevation was found in subjects with evidence of withdrawal symptoms. The authors conclude that several weeks of ethanol ingestion by alcoholics is associated with a marked adrenocortical activation.

If such a stimulation is often *repeated* over long periods of time, it has been reported to result in a state of adrenal cortical and medullary hypofunction and hyperactivity (19 30, 41 42, 49). In man, this presumed hypofunction and hyporeactivity has been hypothesized to increase the craving for continued alcoholic stimulation (46)

In this context it is worth noting that ^{14}C compounds derived from labelled ethanol are reported to accumulate in the liver and kidney but also in the adrenal cortex of rats (24) indicating a relation between ethanol effects and adrenocortical function. However the present body of clinical and experimental evidence is far from sufficient and cannot be said to furnish any definite support for the above hypotheses.

Under these circumstances it seemed worthwhile studying the effect of ethanol ingestion on adrenal cortical and sympathoadrenomedullary function, taking into account not only the immediate effects on the release of adrenal hormones, but also possible after-effects during the week following the experimental treatment.

Some time ago a sedative, chlormethiazole (Heminevrin®), was shown to be a valuable drug

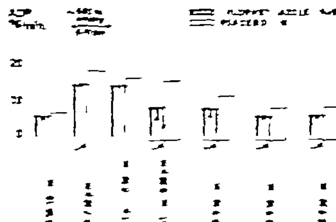


Fig. 1. Urinary adrenaline excretion before, during, and immediately after the ingestion of approximately 500 ml whisky and during Days 2, 4, 6, 8, and 9. Five of the subjects were treated with 0.5 g chlormethiazole at bedtime on the first and with 1.0 g on the second day. Means ± standard errors of the means.

in the treatment of withdrawal symptoms in alcoholics (17). It has also proved effective in the treatment of psychomotor agitation and anxiety states (25, 32, 39). Oral absorption is rapid and most of the drug is excreted in the urine within three hours mainly in the form of methylthiazol-5-acetic acid (2). If the intoxication as well as the withdrawal symptoms actually are related to adrenal mechanisms, it would be interesting to study whether this drug, which is clinically effective against these symptoms, can also modify the concomitant endocrine reactions. Accordingly, as described below, some of the subjects were treated with this drug during and after an experimentally induced episode of ethanol intoxication, and the effects of this treatment on ethanol-induced adrenal cortical and medullary activation were measured.

MATERIAL AND METHODS

Nine healthy male volunteers (medical students), 22–[illegible] years of age, were served 3 g ethanol per kg body weight. This dose, served as and usually amounting to approximately 500 ml of brand whisky, was ingested with food and fluid ad lib. over a period of [illegible] hours ([illegible] p.m.–[illegible] p.m. of Day [illegible]). Five of the subjects were given 0.5 g chlormethiazole at bedtime (at 1.00 a.m. of Day [illegible]) and 1 g on the next evening. The four other subjects were given placebo at the same occasions. The medication was double-blind. Urine samples were obtained before, during, and after ethanol ingestion, as indicated in Figs. 1–[illegible], and analysed for catecholamines according to the method of Euler and Lishajko ([illegible]) and for 17-hydroxycorticosteroids and 17-ketosteroids by the method of Bricke et al. ([illegible]). The morning samples of Days [illegible] (control period) and 4–9 were collected from [illegible] a.m. to 9.30 a.m., during which time the subjects attended routine lectures in internal medicine. In addition, the experiment comprised an "ethanol period" (6.00 p.m. to 7.30 p.m. of Day 1) and a "post-ethanol period" (7.30 p.m. to 1.30 a.m. of the same night, between Days 1 and 2). In order to reduce the risk of drop-outs the period of urine collection on Day 2 was postponed until 11.00 a.m. to 0.30 p.m. ("hang-over period").

As might be expected, all subjects exhibited signs of alcohol intoxication of successively increasing severity as well as a considerable hang-over on the following morning. Although serum ethanol levels were not measured, one may safely assume that measurable amounts of ethanol were still present in the organism also during the hang-over period. During and after the ethanol ingestion, some of the subjects developed a considerable degree of motor activity. Some vomited and behaved in a clearly undisciplined manner. The occurrence of these signs of drunkenness made a strict standardization of the experimental setting unfeasible during the ethanol and post-ethanol periods, although the authors are clearly aware that such standardization is desirable (27).

RESULTS

I. *Adrenaline excretion*

All subjects showed a marked rise in adrenaline excretion during the ethanol (6.00 p.m.–7.30 p.m.) as compared with the control period. The means for our nine subjects during the ethanol and post-ethanol periods show increases by 160 and 143 % respectively from the control period, both increases being statistically significant (Student's t-test, $P < 0.01$) (Fig. 1). Twelve hours later, during the hang-over period, the placebo group excreted significantly higher amounts of adrenaline than the chlormethiazole group ($P < 0.05$). In relation to the control period, the placebo group is still significantly elevated ($P < 0.05$), but this is not the case in the chlormethiazole group. As shown in Fig. 1, this increase in the placebo group gradually disappears during Days 4 and 6, whereas the excretion levels of the chlormethiazole group generally remain close to those from the control

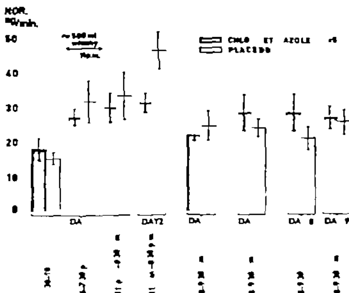


Fig. 2. Urinary noradrenaline excretion during conditions indicated in Fig. 1. Means ± standard errors of the means.

period. The placebo group exhibits a small but significant rise during Day 9 as compared with the level from the control period ($P < 0.05$). The chlormethiazole group shows the same trend but the rise is not statistically significant. For the entire group of nine subjects, the increase during Day 9 over the control level of Day 1 is statistically significant ($P < 0.05$)

II. *Noradrenaline excretion*

Compared with the control period, the noradrenaline excretion of our nine subjects rose 74% during the ethanol period ($P < 0.01$) and 87% during the post-ethanol period ($P < 0.05$). During the hang-over period of Day 2, both groups exhibited a marked increase from the initial control values, by 203% in the placebo group ($P < 0.01$) and by 73% in the chlormethiazole group ($P < 0.05$). The noradrenaline excretion levels of the chlormethiazole group during the hang-over period were significantly lower than those of the placebo group ($P < 0.05$). This significant difference disappeared during Days 4, 6, 8 and 9 (Fig. 2).

III. *17-hydroxycorticosteroid excretion*

No significant increase in mean 17-hydroxycorticosteroid excretion occurred in our nine subjects, either during the ethanol or during the post-ethanol period, although in the latter case there is a trend in that direction, the increase being 37% ($P > 0.05$). However during Days 2, 4 and 6, the excretion levels of the *chlormethiazole* group rose significantly above the initial control values ($P < 0.01$, 0.05 and 0.01 respectively). The *placebo* group shows the same trend, though without ever reaching statistical significance (Fig. 3). During the last two days of our series there is a downward trend in the 17-hydroxycorticosteroid excretion levels of both groups. Comparing the excretion levels of the two groups period by period, we find significant differences during the *control* period only, the placebo group having the higher level ($P < 0.05$).

IV. *17-ketosteroid excretion*

The excretion level did not change significantly in our nine subjects during the ethanol period. During the post-ethanol period, however, there was a significant decrease by 53% ($P < 0.01$). During Day 2, the trend was towards a return to initial levels ($P > 0.05$). During Days 4–9 there was a trend in both groups towards levels somewhat above the initial ones ($P > 0.05$). No significant differences were found between the two groups during any of the periods or days (Fig. 4).

V. *Urine flow*

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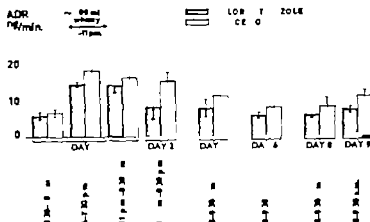


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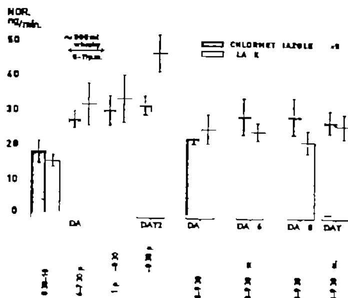


Fig Urinary noradrenaline excretion during conditions indicated in Fig. 1. Means ± standard errors of the means.

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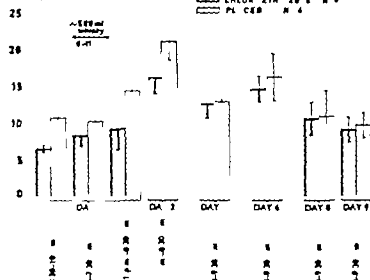


Fig 3 Urinary 17-hydroxycorticosteroid excretion during conditions indicated in Fig. 1 Means ± standard errors of the means.

tween the groups during any of the periods (Fig. 5).

DISCUSSION

The discussion is chiefly concerned with the acute and late effects of ethanol ingestion on the variables studied, including possible hangover effects. The modification, if any of these effects by chlormethiazole is also considered.

I. *Acute Effects of Ethanol Ingestion*

A. *The urinary excretion of adrenaline and noradrenaline*

Our study has demonstrated a pronounced increase in adrenaline and noradrenaline excretion from the control period to the *ethanol* and *post ethanol periods.*

This increase, however does not necessarily imply an ethanol-induced direct or indirect (4) stimulation of the adrenal medulla and the sympathetic nervous system. A number of alternative hypotheses have to be considered.

As has previously been shown (15 27), the urinary excretion of adrenaline and noradrenaline follows a circadian rhythm. If the ethanol ingestion coincided with or was followed by a period during which the catecholamine excretion spontaneously increased, it might be incorrect to attribute the rise to the experimental treatment. This possibility can be ruled out in our case, because

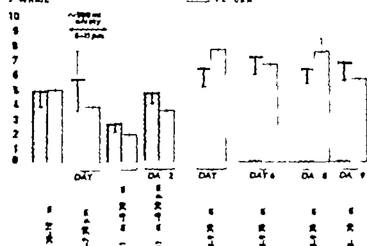


Fig 4 Urinary 17-ketosteroid excretion during conditions indicated in Fig. 1 Means ± standard errors of the means.

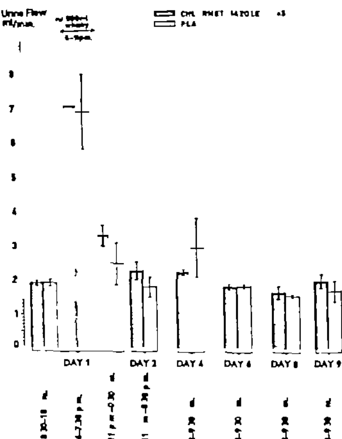


Fig 5 Urine flow during conditions indicated in Fig. 1. Means ± standard errors of the means

the increase occurred during evening hours when the adrenaline excretion normally diminishes quite considerably and the noradrenaline excretion remains unchanged (26).

Several authors have reported a significant, positive correlation between catecholamine excretion and diuresis (22, 35 36, 40) suggesting that the urine volume is the independent variable and the catecholamine excretion the dependent one. The diuretic properties of ethanol are well known. Accordingly the increase in catecholamine excretion could simply reflect a corresponding increase in renal clearance of the catecholamines due to this diuretic action without necessarily reflecting an increased release of these compounds from the sympatho-adrenomedullary system. Comparing the excretion patterns of the free catecholamines and of urine during the control, ethanol, and post-ethanol periods, respectively we find reason to reject this hypothesis. All three variables admittedly exhibit increases of varying magnitude during the ethanol period (Figs. 1 2 and 5) During the post-ethanol period, however the catecholamine excretion remains high, whereas urine flow drops sharply to below the levels during the ethanol period. The correlation coefficients between the changes in urine flow and adrenaline excretion are 0.41 and −0.37 (control minus ethanol and control minus post-ethanol, respectively $P > 0.05$). The corresponding coefficients for changes in urine flow and noradrenaline are 0.15 and 0.24 ($P > 0.05$). Thus, there is no close parallelism between urine flow and catecholamine excretion, the increase in diuresis probably not being responsible for the catecholamine increase found during the ethanol and particularly during the post-ethanol periods.

It is also conceivable that the increase in catecholamine excretion reflects an increased release from storage sites outside the adrenal medulla or an decreased utilization (including O-methylation, oxidation and tissue binding). To our knowledge, this problem has not been adequately studied. Accordingly it cannot be claimed with cer

tainty that the increased catecholamine excretion rates actually do reflect anything but an increased release of these compounds from the sympathoadrenomedullary system. However this may be, the acute increases in catecholamine excretion are marked and statistically significant. Our findings on this point support the reports by other authors, vide supra.

B. *The urinary excretion of 17-hydroxycorticosteroids and 17-ketosteroids*

Neither the 17-hydroxycorticosteroid nor the 17-ketosteroid excretion exhibited any significant changes during the ethanol as compared with the control period. During the post-ethanol period, *there was a non-significant increase by 37% in 17*-hydroxycorticosteroid excretion and a significant decrease by 53% ($P < 0.01$) in 17-ketosteroid excretion. Accordingly, our results do not speak in favour of a *rapid* adrenocortical stimulation induced by ethanol ingestion.

Assuming that the ingestion of large doses of ethanol acts as a stressor, one would expect the adrenal cortex to release increased amounts of its hormones. On the other hand, it is known that the sympathoadrenomedullary system is the first to react during stress, the adrenal cortex being somewhat more sluggish. The trend of 17-hydroxycorticosteroid excretion during the post-ethanol period favours such a hypothesis. True, this increase is not significant. On the other hand it occurred during the time of day when the excretion normally exhibits a decline (1., 48) similar to the one found for 17-ketosteroid excretion. The difference in trends between these two indices of adrenocortical function may be due to the fact that the 17-ketosteroids are derived from the ¹⁹C steroids, which are secreted by both the adrenal cortex *and* the testes, hence the values are influenced by both gonadal and adrenal cortical function, whereas the determination of urinary 17-hydroxycorticosteroids measures the conjugated steroid metabolites derived from the endogenously produced adrenal cortic.. ids, .f which cortisol is the principal substance.

C. *Urine flow*

As shown in Fig. 5, the ingestion of ethanol was accompanied by a very pronounced and highly significant increase in urine flow. During the post-ethanol period, the urine flow was lower but still significantly above the control level. Although the intake of fluid other than ethanol was ad lib, our observations of the subjects' behaviour do not speak in favour of an increased ingestion of non-alcoholic beverages as being the main determinant of this intense diuretic response. The pronounced diuretic effect of ethanol has been discussed by Eggleton (13), Strauss et al. (45) and Heidenreich (18), who explain the phenomenon with reference to an ethanol-induced suppression of the release of antidiuretic hormone from the posterior pituitary gland. However, as the ingestion of non-alcoholic beverages and food was not standardized in our experiment, it cannot be claimed with certainty that this diuretic response actually was *due to the ingestion of ethanol.*

II. *Adrenal Cortical and Medullary Hormone Excretion During Hang-over*

During the "hang-over" period, the placebo group exhibited increased excretion levels of adrenaline and particularly of noradrenaline. These findings are in accordance with those reported by Carlsson and Häggendal (10), who demonstrated increased noradrenaline levels in arterial plasma coinciding with abstinence symptoms upon withdrawal of ethanol in chronic alcoholics. Increased urinary catecholamines have also been reported in such cases by Giacobini et al. (16).

Comparing Figs. 1 and 2, it will be seen that the peak *adrenaline* excretion occurred during the ethanol period, whereas the *noradrenaline* peak did not appear until the hang-over period. In this context it may be relevant to quote the results reported by Klingman and Goodall (23). Working with ethanol intoxicated dogs, these authors found that in addition to a noradrenaline excretion peak between 6 and 9 hours after the alcohol administration, there is a second peak after 21–24 hours. A further indication that a pronounced adrenal medullary stimulation actually had occurred in these dogs was provided by the finding that, 24 hours after the alcohol administration, the adrenaline content of the adrenal glands was 50% below the normal level.

Similarly, there was a pronounced increase in 17-hydroxycorticosteroid excretion during the present hang-over period, whereas the 17-ketosteroid excretion exhibited no change as compared to control levels. As pointed out, the period of urine

collection in Day 2 (hang-over period) started $2\,^1/_2$ hours later than the corresponding period on all the other days. This time difference must be kept in mind when comparing the hormone excretion levels because of the well-known circadian rhythm. On the other hand, the changes were all of such a magnitude that they definitely cannot be explained simply in terms of circadian rhythms for catecholamines (28) and for 17-hydroxycorticosteroids (5). Accordingly our results speak in favour of a marked adrenal stimulation during the hang-over period. It is not clear from the present data whether the increased adrenal hormone levels are due to (*a*) the previous stimulation with ethanol (*b*) the discontinuation of this stimulation (*c*) the remaining levels of ethanol in plasma, or (*d*) the hang-over malaise, possibly including vagotonic as well as sympathotonic activity

III. *Adrenal Function During the Week Following Ethanol Ingestion*

In general, there is a trend towards increased urinary excretion levels of all the hormones studied during Days 4 6 8, and 9 as compared with the control period. This speaks in favour of a prolonged and sustained adrenal cortical and medullary stimulation following ingestion of the large single dose of ethanol. This sustained increased excretion coincides with an elevation of the serum activity of ornithine carbamoyl transferase that was found to occur in a similarly designed experiment (9). A common mechanism for these two reactions remains to be considered.

IV *Chlormethiazole Modification of Ethanol Induced Adrenal Reactions*

As stated above, our data indicate that the catecholamine increases during the "hang-over" period are less pronounced if the subjects have been treated with chlormethiazole. This finding confirms a related result reported by Carlsson and Häggendal (10) indicating that, after chlormethiazole, clinical withdrawal symptoms of a sympathotonic character were mild and that noradrenaline levels in arterial plasma were about normal. A further finding of interest in this context is reported by Allgén et al. (2). Administering chlormethiazole labelled with ^{35}S in the thiazole ring to mice, they found that only one non-excretory organ accumulated a greater amount of radioactivity than the blood namely the adrenal glands.

As to 17-hydroxycorticosteroid excretion, our two groups exhibited different levels already *before* the chlormethiazole medication. For this reason, nothing definite can be said about the subsequent differences in excretion level between the chlormethiazole and the placebo groups.

Thus, our results speak in favour of a modifying effect of chlormethiazole treatment on the sympathoadrenomedullary but not necessarily on the adrenocortical reactions after ethanol ingestion. Whether this effect is a direct one or is due to antiemetic and psychotropic properties of the drug cannot be decided on the basis of the present data.

V *Other Aspects*

Our results suggest that ingestion of a large dose of ethanol induces a stress" reaction in the human organism. This stress reaction *may* be of clinical importance, particularly in two respects. Firstly its frequent recurrence, including peak levels of adrenal hormones, may possibly be detrimental to some organs or organ systems and contribute to the tissue damage found in some chronic alcoholics (6, 11 37 47). Secondly the stressor action of ethanol is often accompanied by the related action of caffeine (15 18) and last but not least of psychosocial influences (29)

Is it conceivable, then, that this combined action, if longstanding, results in a depletion of amine and corticosteroid stores, perhaps even leading to a relative adrenal insufficiency? The question undoubtedly deserves further study

Ethanol, among other effects, impairs man's intellectual, motor and emotional behaviour Further it induces a tendency to produce copious volumes of urine and—as has been shown in the present study—increases in adrenal cortical and medullary function. These changes are perhaps related (21) It is conceivable that part of the mechanism involves a disturbance of water and electrolyte distribution. An earlier study (26) has shown that single doses of opipramol, a minor tranquilizer mainly used in the treatment of neurotic anxiety and depression, induce pronounced diuresis in man. This diuretic response may be considered in the light of the good results reported with this drug in cases of premenstrual tension (20) and possibly also in affective disorders of

tainty that the increased catecholamine excretion rates actually do reflect anything but an increased release of these compounds from the sympathoadrenomedullary system. However this may be, the acute increases in catecholamine excretion are marked and statistically significant. Our findings on this point support the reports by other authors, vide supra.

B *The urinary excretion of 17-hydroxycorticosteroids and 17-ketosteroids*

Neither the 17-hydroxycorticosteroid nor the 17 ketosteroid excretion exhibited any significant changes during the ethanol as compared with the control period. During the post-ethanol period, there was a non-significant increase by 37% in 17 hydroxycorticosteroid excretion and a significant decrease by 53% ($P < 0.01$) in 17 ketosteroid excretion. Accordingly our results do not speak in favour of a *rapid* adrenocortical stimulation induced by ethanol ingestion.

Assuming that the ingestion of large doses of ethanol acts as a stressor one would expect the adrenal cortex to release increased amounts of its hormones. On the other hand, it is known that the sympathoadrenomedullary system is the first to react during stress, the adrenal cortex being somewhat more sluggish. The trend of 17 hydroxycorticosteroid excretion during the post ethanol period favours such a hypothesis. True, this increase is not significant. On the other hand it occurred during the time of day when the excretion normally exhibits a decline (12, 48), similar to the one found for 17-ketosteroid excretion. The difference in trends between these two indices of adrenocortical function may be due to the fact that the 17 ketosteroids are derived from the ^{19}C steroids, which are secreted by both the adrenal cortex *and* the testes hence the values are influenced by both gonadal and adrenal cortical function, whereas the determination of urinary 17 hydroxycorticosteroids measures the conjugated steroid metabolites derived from the endogenously produced adrenal corticoids, of which cortisol is the principal substance.

C. *Urine flow*

As shown in Fig. 5 the ingestion of ethanol was accompanied by a very pronounced and highly significant increase in urine flow During the post ethanol period, the urine flow was lower but still significantly above the control level. Although the intake of fluid other than ethanol was ad lib our observations of the subjects behaviour do not speak in favour of an increased ingestion of non-alcoholic beverages as being the main determinant of this intense diuretic response. The pronounced diuretic effect of ethanol has been discussed by Eggleton (13) Strauss et al. (45), and Heidenreich (18), who explain the phenomenon with reference to an ethanol-induced suppression of the release of antidiuretic hormone from the posterior pituitary gland. However as the ingestion of non-alcoholic beverages and food was not standardized in our experiment, it cannot be claimed with certainty that this diuretic response actually was due to the ingestion of ethanol.

II *Adrenal Cortical and Medullary Hormone Excretion During Hang-over*

During the "hang-over" period, the placebo group exhibited increased excretion levels of adrenaline and particularly of noradrenaline. These findings are in accordance with those reported by Carlsson and Häggendal (10), who demonstrated increased noradrenaline levels in arterial plasma coinciding with abstinence symptoms upon withdrawal of ethanol in chronic alcoholics. Increased urinary catecholamines have also been reported in such cases by Giacobini et al. (16)

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TOTAL HAEMOGLOBIN AND PHYSICAL WORK CAPACITY IN ELDERLY PEOPLE

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Abstract. For a series of clinical healthy subjects of ages 57–71 years, who are considered to fulfil reasonable criteria for representation of the total population, the results are reported of total haemoglobin, blood volume and haemoglobin concentrations, with comparisons between the sexes. Difficulties in calculation of normal values for total haemoglobin and blood volume are discussed. Studies on the relationship between these variables and measures of physical work capacity were made. Results partly deviating from those reported in earlier investigations were obtained. Thus in women no correlation was found between total haemoglobin and blood volume, respectively, and measures of physical work capacity. Possible reasons for this are discussed.

Determinations of total haemoglobin and blood volume are frequently made in both clinical routine work and scientific studies. Interpretation of the information obtained requires access to comparative values, so-called normal values. Numerous reports of such comparative values are available in the literature. Among the figures most often used in Scandinavia are those given by Sjöstrand in 1949 (22), Wiklander in 1956 (27), Holmgren and Strandell in 1959 (12) and Strandell in 1964 (25). Their investigations were performed on series of persons selected according to different principles, and the subjects do not seem to have been representative of the total population. In previous studies a relatively strong, non-age-dependent correlation has been found between blood volume and total haemoglobin, respectively, and physical work capacity (10, 13, 25). Also these studies were based on a selected material, which only with some reservation could be considered representative of the total population, and one of the studies (25) comprised only men. The correlation which was found between total haemoglobin and blood volume, respectively, and physical work capacity may be due to different factors. One of these is the degree of physical fitness of the individual subject. The mean degree of fitness in the community is, however, variable. The rapid structural change in community life during the last decade, which has meant, among other things, that the proportion of the population doing heavy physical work has quickly decreased, has probably resulted in a reduction of the mean degree of physical fitness, with a possibly attendant decrease in the mean values of total haemoglobin and blood volume. It may further have involved a change in the relationship between these variables and physical work capacity.

It therefore seemed important to carry out further studies of the total haemoglobin and blood volume, and the relationship between these variables and physical work capacity, in a group of subjects who could be said to fulfil reasonable requirements for representation of the total population.

It was considered of especial value to study first and foremost older men and women, since certain earlier studies have comprised mainly young and middle-aged persons of both sexes, and since studies which have included older persons have only comprised men. Sex comparisons in elderly persons on the basis of early reports therefore seem unreliable. My own previous studies of the effect of ageing on the ventilatory capacity and physical work capacity, for which the results deviated partly from earlier reports, were also a motivation for the present investigation (4, 5).

MATERIAL

The principles followed in selection of the subjects have been described previously (3). There were some exclusions from the original material in later determinations

of the physical work capacity total haemoglobin and static lung volumes, and details of these exclusions have been given in previous paper (4). In the present study two subjects ere excluded when, in the physical work test, there was some reason to suspect coronary insufficiency. Values for total haemoglobin and blood volume are lacking for tw other men (one because of refusal, and the other because of inability to participate). Values for total haemoglobin are lacking for four women (who refused to participate) and for haemoglobin concentration for one woman (the value obtained was clearly erroneous and was therefore excluded from the results). Thus blood volume alues are lacking for altogether five women

The subjects were divided into the following age groups: 57–61 years (17 men, 18 women), 62–66 years (17 men, ..3 women) and 67 71 years (12 men, 10 women). Certain anthropometric data for this series have been presented in previous paper (4). The minor deviations in the composition *of the material*, described above, had no noteworthy influence on these data, which are thus in essential greement with the corresponding data for the complete series.

METHODS

All examinations were performed during the morning on tw consecutive days. The patients were asked not to smoke fter 5 p m the previous day. Three of 46 women and 26 of 44 men were smokers. On the first day the determination of total haemoglobin and haemoglobin concentration as followed by lung function tests and, finally physical work tests. On the second day the total haemoglobin and haemoglobin concentration were determined at the same time as on the previous day

Total haemoglobin

The total haemoglobin was determined by the alveolar CO met d. The direct examination of the subject was perform d according to Sjöstrand (21), with the modification tha the subject rebreathed in bag for 30 min after the administr tion of CO. The administered volume of CO (99.5%) ried between 20 and 30 ml depending on the body weight and relativ haemoglobin value of the subject.

The analyses ere performed according to the method of Linderholm and Sjöstrand (16), with modifications of the apparatus according to Linderholm (14).

Duplicate determinations were made in the majority of subjects. The error of he method ($\sigma = \pm\sqrt{\Sigma d^2/2n}$) calculated on 39 and 36 duplicate determinations in men and women, respectiv ly as ±36.98 g for men and ±25.08 g for women, cor esponding to 5.35% and 4.50% of the mean values for the respectiv sexes.

For calculation of the total haemoglobin a simplified, approximative formula, the so-called aproximate alue formula (15), was used. Previous investigations have shown that calculations according to this simplified formula have agreed well (±..00%) ith those in hich the original formula was used (25)

The room temperature as ... °C. Since previous studies have show that the temperature factor is 1.000 at ± 1.6°C, and only varies by ±0.0030 at a temperature variation of ±1.0° it has been considered negligible (26). The volume of the rebreathing apparatus amounted to about 3 L. For about the first half of the series, balloons with a volume of 7 l were used, and for the second half balloons with volume of 5 L. The equation used was thus as follows:

$$THb = \frac{2.66 \quad V \quad F_{aco} \quad F_{e_2} \quad K}{1000 \quad (F_{co_{II}} - F_{co})}$$

where

- V = volume of concentrated CO gas supplied to the system (20–30 ml ATP (Methods, paragraph ?)
- F_{aco} = fraction of CO in the gas supplied to the system = 0.995
- F_{e_2} = fraction of O in the second sampling robber bag
- F_{co_I} = fraction of CO in the first sampling rubber bag before the supply of CO
- $F_{co_{II}}$ = fraction of CO in the second sampling rubber bag 30 min after supply of CO
- K = correction factor for the barometric pressure. The slope of the factor on P was positive. At 760 mm Hg the facto was 1.000. For change of ±10 mm Hg it changed by ±0.0138.

When the 7 l balloon was changed to a 5 l balloon the factor 2.66 was changed to 2.60.

Haemoglobin concentration

The haemoglobin concentration (Hb conc., g/100 ml) was measured spectrophotometrically as cyanmethaemoglobin, using Acuhne solution (Ortho Pharmaceutical Corp., New Jersey U.S.A.). The determinations were made on capillary blood from the finger-tip taken after the subject had been sitting for at least 30 min. Samples were taken on two consecutive days. On each occasion, with only few exceptions, duplicate analysis was performed. The mean value for all determinations was calculated.

The error of the method ($\sigma = \pm\sqrt{\Sigma d^2/2n}$) for Hb conc., calculated on 35 and 30 duplicate determinations from tw consecutive days for men and women, respectively was 0.436 g% for men and 0.660 g% for women, corresponding to 3.29 and 5.30% of the mean value for the respective sexes. The error of the method calculated on all analyses (i.e. 1..6 and 108 for men and women, respectively) was 0.010 extinction unit for men, corresponding to relative scatter ($\sigma/\bar{x}$ 100) of 2.2 .. nd 0.008 xtinction unit for women, or 1.9%

Blood olume

The total blood volume as obtained as the ratio between total haemoglobin and haemoglobin concentration, with correction for the difference between body haematocrit and haematocrit in capillary finger blood (8), according to the equation

$$TBV\ (\text{litres}) = \frac{THb\ (g)}{9.1 \quad Hb\ conc.\ (g \quad)}$$

The error of the method ($\sigma = \pm\sqrt{\Sigma d^2/2n}$) was 0.368 l for men and 0..45 l for women, calculated on 38 and 35 duplicate determinations, respectively corresponding to 6.37%, and 4.93% f the mean value for the respective sexes

Table I *Mean value standard deviation and standard error of the mean for total haemoglobin (THb), haemoglobin concentration (Hb conc.) and total blood volume (TBV) after grouping with regard to sex and age*

		THb (g)				Hb conc. (g)				TBV (l)			
		Mean	S.D.	S.E.	S.D. 100 / Mean	Mean	S.D.	S.E.	S.D. 100 / Mean	Mean	S.D.	S.E.	S.D. 100 / Mean
Men													
I	57–61	720.1	70.89	17.19	9.8	13.6	0.84	0.20	6.2	5.9	0.68	0.17	11.5
	No. 17	17				17				17			
II	62–66	664.7	89.38	22.35	13.4	13.1	0.75	0.18	5.7	5.6	0.84	0.21	15.0
	No. 17	16				17				16			
III	67–71	705.3	113.67	34.27	16.1	13.4	0.97	0.29	7.2	5.9	0.92	0.28	15.6
	No. 12	11				11				11			
Total		696.2	90.98	13.72	13.1	13.3	0.85	0.13	6.4	5.8	0.80	0.12	13.8
	46	44				45				44			
Women													
I	57–61	551.8	77.99	18.38	14.1	12.5	0.64	0.15	5.1	4.9	0.66	0.16	13.9
	No. 18	18				18				18			
II	62–66	546.6	76.90	17.64	14.1	12.8	0.69	0.16	5.4	4.8	0.72	0.17	15.0
	No. [illegible]3	19				18				18			
III	67–71	519.1	72.91	24.30	14.0	12.3	0.75	0.25	6.1	4.7	0.73	0.[illegible]4	15.5
	No. 10	9				9				9			
Total		543.3	75.88	11.19	14.0	12.6	0.70	0.10	5.5	4.8	0.69	0.10	14.4
	51	46				45				45			

RESULTS

For the results of the physical work test, the reader is referred to a previous paper on physical work capacity and static lung volumes in the elderly (4). Because of the exclusion of two subjects from the present study new calculations of the mean values for the different age groups were performed and only very slight deviations were found.

Table I gives the mean values, standard deviation, standard error of the mean and standard deviation in per cent of the mean value for total haemoglobin (THb), haemoglobin concentration (Hb conc.) and blood volumes (TBV) for both men and women, grouped according to age.

Table II gives the corresponding data for haemoglobin per kg body weight (Hb/kg) and blood volume per kg body weight (BV/kg).

The mean value for Hb conc. was 13.3% in men and 12.6% in women, thus 5.3% lower in women than in men ($p < 0.001$). The corresponding figures for THb were 696 and 543 g, respectively thus on the average 22% lower in the women ($p < 0.001$). For TBV the corresponding values were 5.8 and 4.8, respectively thus 17.2% lower in the women ($p < 0.001$). Even when consid-ration was taken of the sex difference in body size, as reflected by body weight, the women still showed lower values. The mean value for Hb/kg was 9.6 g/kg for men and 8.1 g/kg for women, thus 15.6% lower in women than in men ($p < 0.001$). The corresponding values for BV/kg were 80.5 and 72.2 ml/kg, and 10.3% respectively ($0.001 < p < 0.01$). Between the different age groups there were only minor non-significant differences, which showed no tendency to age dependence.

Table III gives the correlation coefficients between THb, Hb conc., TBV and age, height and weight. Apart from the obviously strong correlation between THb and TBV the correlations were relatively weak throughout. As expected, THb and TBV were positively correlated to height and weight, the correlation to weight was, as a rule, somewhat stronger than that to height. A positive but only very weak correlation was found between Hb conc. and THb.

As will be seen from Table I, for Hb conc. the standard deviation in per cent of the mean value was 6.4 for men and 5.5 for women. The corresponding figures for THb were 13.1 and 14.0% and for TBV 13.8 and 14.4% respectively. Thus

Table II. *Mean value standard deviation and standard error of the mean for haemoglobin amount and blood volume per kg body weight after grouping with regard to sex and age*

		Hb/kg (g/kg)				BV/kg (ml/kg)			
		Mean	S.D	S.E.M	S.D 100 / Mean	Mean	S.D	S.E.M.	S.D 100 / Mean
Men									
I	57–61	9.9	1 41	0.34	14.2	81.0	11.53	2.80	14.2
	No. 17	17				17			
II	62–66	9.0	1.24	0.31	13.8	76.6	12.17	3 04	15.9
	No 17	16				16			
III	67 71	10.1	1.52	0.46	15.0	85.3	16.47	4.07	19.3
	No 12	11				11			
Total		9 6	1 43	0.21	14.9	80.5	13.27	2.00	16.5
	46	44				44			
Women									
I	57–61	8.2	1.27	0.30	15.5	73.2	11 11	2.62	15.2
	N 18	18				18			
II	62–66	8.0	1.33	0.31	16.6	70.6	12.25	2.89	17 4
	N 23	19				18			
III	67–71	8.2	1 19	0 40	14.5	73.4	9.88	3.29	13.5
	No 10	9				9			
Total		8.1	1.26	0 19	15.5	72.2	11 19	1.67	15.5
	51	46				45			

the standard deviation for both these latter variables was large. Since both THb and TBV are functions of body size, it is possible that the relatively large scatter may be due to the variation in body size It is evident from Table II, however that the scatter was still wide even when the variation in body weight was taken into consideration. Thus it does not seem possible to calculate THb and TBV with accuracy on the basis of body weight alone.

With the aim of finding out which combination of independent variables permits the most reliable calculation of the normal value for THb and TBV a multiple linear regression analysis was performed. Table IV gives a selection of results from different regression equations with THb as dependent variable, and Table V the results from corresponding regression equations with TBV as dependent variable

Total haemoglobin

It will be seen from Table IV that, in all regression approaches with THb as dependent variable a somewhat higher explanatory value was attained for men than for women. Body weight as the only independent variable showed for both sexes a

Table III. *Correlation coefficients with significance asterisks, between total haemoglobin (THb), haemoglobin concentration (Hb conc), total blood volume (TBV) and age body height and body weight and between Hb conc and THb and TBV*

	Sex	Age	Height	Weight	TBV	THb
THb	♂	0 169	0 429*	0.459*	0 876	
	♀	0 181	0 319*	+0.388	+0 885	
Hb conc.	♂	0 235	0 112	0 007	−0.328	+0.151
	♀	0.004	0 102	0 034	−0.284	0.128
TBV	♂	−0 008	0 456	+0.413		
	♀	−0.288	0 277	+0.457**		

0.01 < p ≤ 0 05   0 001 p ≤ 0 01   0.001 p

Table IV *Total haemoglobin (g) in relation to age height, weight and haemoglobin concentration. The asterisks represent degrees of significance*

Regression equations	Sex	Constant term	Regression coefficients: Age (y)	Height (cm)	Weight (kg)	Hb conc. (g%)	R	Residual S.D: g	% of mean
1	♂	−207	—	+5.16** (±1.81)	—	—	0.403	84.2	12.1
	♀	−118	—	+4.02* (±1.82)	—	—	0.317	72.8	13.4
2	♂	+394	—	—	+4.14 (±1.17)		0.478	80.9	11.6
	♀	+287	—	—	+3.78** (±1.05)	—	0.476	67.5	12.4
3	♂	+80	−4.45 (±3.15)	+4.08 (±1.93)	+2.49 (±1.31)	—	0.554	77.4	11.2
	♀	−117	−1.46 (±2.79)	+3.48* (±1.71)	+2.66 (±1.16)	—	0.484	67.4	12.4
4	♂	−208	−3.60 (±3.25)	+4.23* (±1.93)	+2.46 (±1.31)	+15.80 (±15.37)	0.571	77.4	11.2
	♀	−147	−1.84 (±2.60)	+2.52 (±1.63)	+3.25** (±1.10)	+13.98 (±13.92)	0.535	62.8	11.5

$0.01 < p \leq 0.05$ ** $0.001 < p \leq 0.01$ *** $0.001 \geq p$

somewhat higher explanatory value than body height (R ♂ = 0.478 and 0.403 and R ♀ = 0.476 and 0.317 respectively). When height and weight were combined, a slightly higher explanatory value was obtained for both sexes (R ♂ = 0.521 and R ♀ = 0.478) than when body weight was the only independent variable. Fig. 1 illustrates the correlation between THb and weight, Fig. 3 the importance of weight for THb at a given height. On the introduction of ages as a further independent variable together with height and weight, only a negligibly increased explanatory value was obtained (R ♂ = 0.554 and R ♀ = 0.484). With regard to the importance of Hb conc. as an independent, explanatory variable, there was a tendency to a difference between the sexes. For

Table V *Blood volume (l) in relation to age height weight and haemoglobin concentration. The asterisks represent degrees of significance*

Regression equations	Sex	Constant term	Regression coefficients: Age (y)	Height (cm)	Weight (kg)	Hb conc. (g%)	R	Residual S.D: (l)	(% of mean)
1	♂	−3.04	—	+0.051 (±0.015)	—	—	0.451	0.72	12.4
	♀	−0.86	—	+0.035* (±0.017)	—	—	0.303	0.66	13.8
2	♂	+3.50	—	—	+0.031 (±0.011)	—	0.416	0.73	12.6
	♀	+2.41	—	—	+0.036** (±0.009)	—	0.499	0.60	12.5
3	♂	−1.89	−0.008 (±0.029)	+0.039* (±0.018)	+0.019 (±0.012)		0.512	0.72	12.4
	♀	+0.92	−0.029 (±0.024)	+0.024 (±0.015)	+0.027* (±0.010)		0.531	0.58	12.1
4	♂	+4.08	−0.026 (±0.028)	+0.036* (±0.017)	+0.020* (±0.011)	−0.327* (±0.134)	0.601	0.67	11.6
	♀	+3.73	−0.029 (±0.023)	+0.027 (±0.014)	+0.027* (±0.010)	−0.269* (±0.123)	0.602	0.55	11.6

$0.01 < p \leq 0.05$ ** $0.001 < p \leq 0.01$ *** $0.001 \geq p$

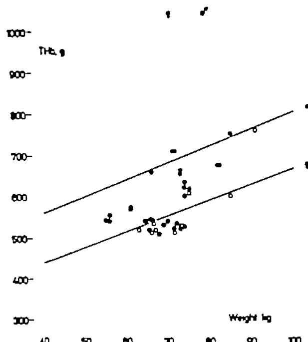


Fig 1 Total haemoglobin in relation to body weight. Regression lines for men and women indicated. O = women. ● = men.

the women R increased from 0.484 to 0.555 but for the men only from 0.554 to 0.571 The coefficient of regression for Hb conc. was not significant, however for either sex. The lowest residual standard deviation obtained with any of the tested regression approaches was 77.4 g for men and 62.8 g for women, corresponding to 11.2 and 11.5 % of the mean value for the subjects included in the regression approach.

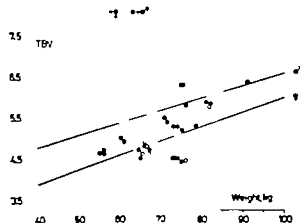


Fig 2 Blood volume in relation to body weight. Regression lines for men and women indicated. O = women. ● = men.

Blood volume

It is evident from Table V that for the women, body weight as the only independent variable had a higher explanatory value than body height (R ♀ = 0.499 and 0 303 respectively), while for the men weight and height had approximately the same explanatory capacity (R ♂ = 0 416 and 0 451 respectively) When height and weight were combined, a somewhat higher explanatory value was obtained for both men and women (R ♂ = 0.510 and R ♀ = 0.504). Fig. 2. illustrates the correlation between TBV and weight Fig. 4 the importance of weight for TBV at a given height. The introduction of age as a third independent variable together with height and weight gave no appreciable increase in the explanatory value of the regression approach (R ♂ = 0.512 and R ♀ = 0.531). The residual standard deviation was 0.72 l for men and 0.58 l for women corresponding to 12.4 and 12.1 % respectively of the mean value for subjects included in the regression approach. When Hb conc. was introduced as a further independent variable, a higher explanatory value was obtained (R ♂ = 0.601 and R ♀ = 0.607). On evaluation of the effect of Hb conc. on TBV however consideration must be paid to the fact that TBV was not determined by an independent method but was calculated as the ratio between THb and Hb conc.

To summarize, it may be said that THb and TBV cannot be calculated with a particularly high degree of accuracy from any of the tested combinations of independent variables in a material of the present type.

Table VI gives the correlation coefficients between measures of physical work capacity and total haemoglobin (THb), blood volume (TBV)

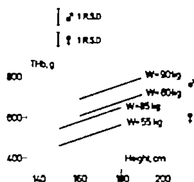


Fig 3 Nomogram illustrating the influence of weight on THb.

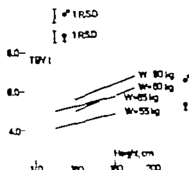


Fig. 4. Nomogram illustrating the influence of weight on TBV.

haemoglobin per kg body weight (Hb/kg), and Hb conc. For the women very weak correlations were obtained throughout between these measures of physical work capacity and THb and TBV respectively. When the haemoglobin mass was expressed as concentration values (Hb/kg, Hb conc.), the correlations were still equally low. For the men considerably higher correlation coefficients were obtained between measures of physical work capacity and THb and TBV respectively and the correlation with TBV was higher than that with THb. When the haemoglobin mass and the blood volume were expressed as concentration values, low and in some cases negative correlation coefficients were obtained throughout.

DISCUSSION

The total blood volume (TBV) is in normal cases adapted to the volume of the vascular system and thus first and foremost a function of body size but not directly dependent upon the haemoglobin concentration. This can also be expressed as follows—that, even if the body has the capacity for rapidly adapting TBV to the circulatory demand by changing the plasma volume, e.g. on bleeding, this capacity is not utilized to an appreciable degree under normal conditions. The total haemoglobin mass (THb) is similarly under normal conditions, mainly a function of the volume of the vascular system, but also a function of the haematopoietic activity. THb can thus be expected to be positively correlated to Hb conc. In a series of subjects which does not include persons with manifest anaemia the variation in Hb conc. is, however, relatively low, while the variation in body size may be considerable. Under such circumstances no strong correlation between THb and Hb conc. should be expected.

With increasing degree of physical fitness there is an increase in THb, at least in very fit persons. A decrease in Hb conc. with increasing degrees of fitness has also been found (9), but the reason for this is unclear. One important factor may be the rapid adaptation of TBV to the circulatory demand by means of an increase in the plasma volume. The degree of physical fitness thus seems to be a factor which under certain conditions may give rise to a negative correlation between THb and Hb conc.

Strandell (25) found a relatively strong positive correlation between THb and Hb conc. in men of ages 30–83 years ($r = 0.433$ at $n \approx 70$ and regression coefficient significant with $p \leq 0.001$). In the present study a positive but weak correlation was found between THb and Hb conc. in both

Table VI. *Correlation coefficients—with significance asterisks—between indices of physical work capacity and total haemoglobin (THb), blood volume (TBV), amount of haemoglobin per kg body weight (Hb kg), and haemoglobin concentration (Hb conc.)*

	Sex	No.	THb	TBV	Hb conc.	Hb/kg
W[illegible]	♂	39	0.364	0.511**	−0.300	0.162
	♀	37	0.128	0.098	0.150	0.129
W[illegible]	♂	39	0.385*	0.514**	−0.259	0.169
	♀	34	0.140	0.179	0.111	0.042
W[illegible]	♂	25	0.302	0.421	−0.166	0.079
	♀	18	0.046	−0.022	0.173	−0.095
W[illegible]	♂	40	0.341	0.473**	−0.281	0.172
	♀	37	0.066	0.063	0.083	−0.075

$0.01 < p \leq 0.05$. ** $0.001 < p \leq 0.01$. *** $0.001 > p$.

men and women. The multiple regression analysis with THb as dependent variable showed that in men Hb conc. as the independent variable did not increase the explanatory value in any of the tested regression approaches, and that the coefficients of regression were very low throughout ($p > 0.05$). For the women, on the other hand, the introduction of Hb conc. as a further variable seemed in all regression approaches to result in a slightly higher explanatory value: the regression coefficient was never significant, however ($p > 0.05$). The suggestion of a sex difference in this respect might have been due to the fact that in women, to a greater extent than in men, THb is limited by the capacity of the haematopoietic system, since the variation in body size was considerable in both sexes. The sex difference might also be connected with variations in degree of physical training.

A close analysis of the importance of the age factor for THb and TBV requires a material with a considerable age range. This condition was not fulfilled satisfactorily in the present investigation. A negative but very low correlation was found between age and THb and TBV respectively. In the regression anlyses, however the introduction of age as an independent variable, after consideration had been taken of body height and weight, did not result in any noteworthy increase the explanatory value, and the regression coefficients were not significant. This indicates that the age factor in itself is not of such importance that it has any influence within the age range covered by this investigation.

Previous studies have shown contradictory results in this respect. Some authors have found that the total haemoglobin decreases with increasing age (1 6, 17 –3, –5), while others have found that in adults the total haemoglobin is not age-dependent when consideration is paid to variations in body height and weight. In the studies referred to, both the methods and the principles for selection of subjects varied. In some investigations the number of elderly subjects was relatively small. In a number of studies no consideration was paid to the reduction in height and weight with increasing age. Such factors may explain the divergent results.

In several investigations a high correlation has been found between measures of physical work capacity and, respectively total haemoglobin (THb) and blood volume (TBV) (10 11 12, 13, 25). It seems reasonable also that, at least in younger persons with a good adaptation capacity of the heart and vascular system, such a correlation should be found between physical work capacity and variables constituting measures of the volume of the vascular system. Thus even if, in principle, such a correlation exists, several other factors may influence and, under certain conditions, be assumed to be of great importance for the physical work capacity. Variations in the way in which available haemoglobin is distributed between different organic systems is one such possible factor. Thus there may be individual variations in the capacity for distributing blood to the working muscles during physical work. It also seems reasonable to assume that, for example, at higher ages, the ability of the vascular system to increase its volume in connection with physical training decreases, and that the increase in the physical work capacity is rendered possible by an increase in the arterio-venous oxygen difference. It has in fact been shown previously that the decrease in stroke volume with increasing age is compensated by an increase in the arterio-venous oxygen difference (7). It may also probably be assumed that, with decreasing and sufficiently low degrees of physical fitness, the physical work capacity decreases more than the total haemoglobin and blood volume. In all of these outlined situations the correlation between measures of physical work capacity and THb or TBV would tend to decrease.

For these latter correlations the present study showed results which partly deviated from those published previously. For men there was a positive but relatively low correlation between the variables constituting measures of physical work capacity (W_{130}, W_{150}, W [illegible] and W_{max}) and THb and TBV respectively. For women no such correlation was found. This sex difference was probably due to the fact that the women in the series had a considerably lower degree of physical fitness than the men, and that with decreasing degree of fitness THb and TBV are not reduced to the same extent as the physical work capacity.

The correlation coefficients between the variables comprising measures of physical work capacity and Hb conc. were low for both sexes. It is probable that these correlations were due to chance and should not be attributed any biological importance.

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PLASMA GROWTH HORMONE AND HYPERTROPHIC OSTEOARTHROPATHY IN CARCINOMA OF THE BRONCHUS

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Abstract. Three cases of hypertrophic osteoarthropathy (Pierre Marie–Bamberger syndrome) with co-existing carcinoma of the bronchus are reported. Estimations of plasma growth hormone including the variations during hypo- and hyperglycemia are undertaken pre- and post-operatively. In one case the fasting plasma growth hormone concentration was elevated, presenting characteristics suggestive of autonomous secretion. The clinical picture of hypertrophic osteoarthropathy to some extent mimics that of acromegaly but the evidence that hypertrophic osteoarthropathy is the result of increased production of growth hormone is not found to be convincing. It is concluded that cells from malignant lung tumors might be able to synthesize and secrete growth hormone or growth-hormone-like substances.

The production of hormones by tumors arising from organs that do not normally synthesize hormones is now a well established paraneoplastic manifestation. The ectopic hormone production produces clinical syndromes that closely mimic those due to hyperfunction of a particular endocrine gland. In the ectopic A.C.T.H. syndrome the polypeptide is in some cases probably identical to pituitary A.C.T.H., but in other of the syndromes the structure of the substances with hormonal activity is unknown (21).

The pathogenesis of the clinical entity of hypertrophic osteoarthropathy (HOA) and lung cancer (Pierre Marie–Bamberger syndrome) is not established. Many hypotheses have been proposed to explain the development of osteoarthropathy but no single explanation has been generally accepted (19). Several authors have been impressed by the clinical similarity between acromegaly and osteoarthropathy (17, 18, 23, 26). In 1968 Steiner et al. (26) described a patient with carcinoma of the bronchus, osteoarthropathy and elevated plasma levels of human growth hormone (HGH) which normalised after resection of the carcinoma. They suggested ectopic production of HGH by the pulmonary tumor and raised the question of hypertrophic osteoarthropathy and lung cancer as an ectopic HGH syndrome.

We report here on three patients with carcinoma of the bronchus and osteoarthropathy in which HGH determinations were performed.

CASE REPORTS

Case 1

A 54-year-old woman who has been well until May 1968. At this time she developed stiffness, pains and edema of the hands, feet and crura. There was weight loss of five kg during four months, but no pulmonary symptoms. The sedimentation rate was 80 to 100 mm/h, and she was admitted to the Medical Department A, Rigshospitalet, in August 1968.

On admission the patient appeared chronically ill and her coarsened facial features were suggestive of acromegaly. The physical examination was normal except for the extremities. There was edema of the crura, feet and hands, marked clubbing of the fingers and toes and tenderness of the long bones.

The X-ray of the chest demonstrated a coin lesion in the left upper lobe three cm in diameter. At the mediastinoscopy no metastases to the lymph nodes were demonstrated. The X-ray of the long bones, metacarpal, metatarsal and phalanges of the hands and feet revealed marked periosteal proliferations (hypertrophic osteoarthropathy) (Fig. 1). The X-ray of the skull and the sella turcica was normal.

Laboratory data. Hb 10.4 g/100 ml, sedimentation rate 86 mm/h, serum-creatinine 0.7 mg/100 ml, serum-GP transaminases 0.4 U/ml (normal ≤1.5 U), serum-bilirubin 0.4 mg/100 ml, bromsulfalein retention test normal, serum-calcium 8.5/100 ml (normal 9.4–10.6 mg/100 ml), ionized serum-calcium 6.35 mg/100 ml (normal 6.00–6.60 mg).



Fig 1 Periosteal proliferations on the metatarsals and phalanges from a 54-year old woman (case 1).

alkaline phosphatases in serum 98 U/ml (normal 35 U).

Fasting blood glucose level 93 mg/100 ml, oral glucose tolerance test normal.

Urine 17 KS and 17 KGS normal excretion in 24 h. Plasma hydrocortisone 21.9–20.0 μg/100 ml plasma (at 8 a.m. and 8 p.m., respectively)

On the 26th August, 1968, a left upper lobe lobectomy was performed (histological diagnosis of the lung tumor adenocarcinoma). Postoperatively the pains and stiffness of the extremities decreased, and the edema disappeared

Case 2

A 69-year-old man who was admitted to Surgical Department R, Rigshospitalet, in November 1968.

Except for chronic bronchitis the patient had been well until June 1968. From then on he developed severe edema of the crura, feet and hands, resistant to diuretic treatment (Figs. 2 and 3). The fingers and toes gradually became clubbed, and the patient was rendered immobile because of stiffness and pains in the extremities.

There was no weight loss, and the pulmonary symptoms did not progress.

The physical examination revealed severe edema of the crura, hands and feet, clubbing of the fingers and toes, and tenderness of the long bones. No acromegalic features were observed.

X-ray of the chest revealed a tumor in the left lung 5 cm in diameter. On mediastinoscopy small metastases were demonstrated in a single lymph node. The bronchoscopy was normal.



Fig 2. Edema of the crura and feet, and clubbing of the toes in a 69-year-old man (case 2).

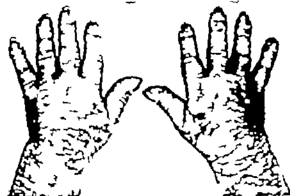


Fig. 3 Edema of the hands and clubbing of the fingers in a 69-year-old man (case 2).



Fig. 4. Periosteal proliferations, especially around the joints in 69-year-old man (case 2).

X-ray of the long bones, hands and feet demonstrated periosteal proliferations, especially around the joints (hypertrophic osteoarthropathy) (Fig. 4). The X-ray examination of the skull and sella turcica was normal.

Laboratory data. Hb 11.8 g/100 ml, sedimentation rate 66 mm/h, serum-bilirubin 0.5 mg/100 ml, serum-GP transaminases 0.9 U/ml, alkaline phosphatases in serum 32 U/ml, serum-creatinine 1.. mg/100 ml.

Fasting blood glucose level 84 mg/100 ml, oral glucose tolerance test, normal. Plasma hydrocortisone 17.3 μg/100 ml, urine 17 KS and 17 KGS, normal excretion.

In spite of metastases to mediastinal lymph nodes and because of the severe symptoms from the extremities left-sided pneumonectomy was performed (histological diagnosis of the lung tumor carcinoma planocellulare parakeratoticum).

The symptoms from the extremities subsided dramatically postoperatively and the edema disappeared.

Case 3

A 67-year-old man admitted to Medical Department, Københavns Amts Sygehus i Lyngby in January 1969. For eight years he has had symptoms of chronic bronchitis, progressing during the last few months. In the last year he had increasing edema of the crura and feet, and two months before admission he developed stiffness, slight pains in the hands and feet, and edema resistant to diuretic treatment. There was weight loss of two kg in three months.

The physical examination demonstrated slight edema of crura, feet and hands. There was clubbing of the fingers and toes, but no tenderness of the long bones. No signs of acromegaly.

The chest X-ray demonstrated coin lesion measuring two cm in diameter, localized in the periphery of the right lung. Bronchoscopy was normal, while metastases to the lymph nodes were found at mediastinoscopy (histological diagnosis: carcinoma solidum).

The X-ray of the long bones, hands and feet demonstrated hypertrophic osteoarthropathy. The X-ray of the skull and sella turcica was normal, and the eye examination normal.

Laboratory data. Hb 16.6 g/100 ml, sedimentation rate 9 mm/h, serum-GO-transaminases 10 U/ml (normal < 40 U). Serum-creatinine 1.6 mg/100 ml. Urine: no protein, glucose or blood. Plasma corticosteroid: 20.2 μg 100 ml (at 9 a.m.).

Fasting blood sugar level 185–198 mg/100 ml. Oral glucose tolerance test was diabetic. Fasting plasma insulin was elevated, 57 microunits/ml.

Because of the slight symptoms from the extremities, and the findings of metastases, the patient was not operated upon.

PLASMA GROWTH HORMONE STUDIES

The plasma human-growth hormone (HGH) studies were performed on blood samples collected in heparinized tubes. The patients had been fasting for at least 1.. h and were resting. The samples were centrifuged for 15 min at 3 000 r.p.m., and the plasma frozen within 30 min at −20°C until use. Standard insulin tolerance tests were carried out after 1.. h overnight fasting. Crystalline insulin, 0.1 u. per kg body weight, diluted in 10 ml of physiological saline solution, was given intravenously and blood was sampled for determination of glucose and HGH every 10–15 min for 60 to 90 min (8). Oral glucose tolerance test (70 g glucose peroral) with determination of blood sugar and plasma HGH was performed in two of the patients (cases 2 and 3). In one patient (case 3)

Table I. *Plasma HGH in three patients with hypertrophic osteoarthropathy and carcinoma of the bronchus*

			Fasting plasma HGH	
Case	Sex	Age	Preoperatively	Postoperatively
1	♀	54	4.5	7.6
2	♂	69	5.7	6.2
3	♂	67	20	—

plasma insulin was estimated during oral glucose tolerance test.

The plasma HGH was determined by double antibody immunoassay. The method described by Hales and Randle (16) for the assay of insulin in plasma as modified by Brunfeldt and Jørgensen (4) was applied to HGH in plasma. Iodination was performed according to Greenwood and Hunter (13) with slight modifications (12), using J^{131} as tracer and highly purified HGH prepared by P. Fønss-Bech, Department of Biochemistry, Nordisk Insulin Laboratory, Gentofte, Denmark (10). This preparation was also used for the immunization of guinea-pigs. Normal fasting levels in our laboratory ranged between 1.5–8.0 mμg/ml plasma ±10% (Plasma pool, mean 6.6 mμg/plasma, S.D. ± 0.6 mμg.)

Plasma insulin concentrations were measured as immunologically detectable insulin by the method of Hales and Randle (16) as modified by Brunfeldt and Jørgensen (4), using J^{131}-insulin as tracer. Normal fasting values: 6–26 microunits/ml plasma.

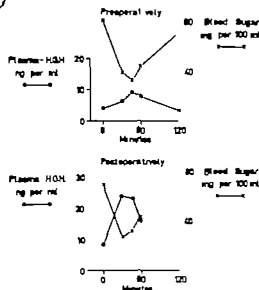


Fig. 5. Standard insulin tolerance tests in a 54-year-old woman (case 1) demonstrating subnormal increase in plasma HGH preoperatively and normal response postoperatively.

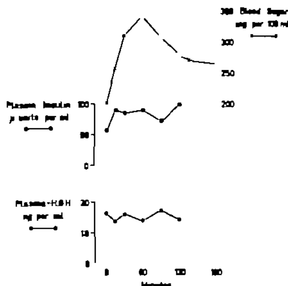


Fig. 6. Plasma insulin and plasma HGH during peroral glucose tolerance test in 67-year-old man (case 3). The *glucose tolerance test is diabetic, the plasma insulin* elevated, and the plasma HGH elevated and unchanged during the test.

RESULTS

The results of the plasma HGH determinations in the three patients are summarized in Table I. In two patients (cases 1 and 2) the fasting plasma HGH values were normal before and after the operation of the lung tumor. In one patient (case 2) there was normal plasma HGH response to hypoglycemia and hyperglycemia preoperatively. Case 1 had a subnormal increase in plasma HGH concentrations following insulin-induced hypoglycemia preoperatively while it was normal after removal of the tumor (Fig. 5).

In case 3 the fasting plasma HGH concentration was increased (20 mμg/ml plasma). There was no plasma HGH response to insulin-induced hypoglycemia. But the decrease in blood sugar was only 30%. Because of the patient's poor general condition another insulin tolerance test could not be performed. However there was no decrease in plasma HGH values during the glucose tolerance test. This patient had elevated fasting blood sugar concentration (198 mg/100 ml) and the glucose tolerance test was diabetic. The fasting plasma insulin concentration was elevated (57 microunits/ml) and the plasma insulin level increased to 72–96 microunits/ml, remaining on this high level 120 min after glucose intake, while the blood sugar was 308–277 mg/100 ml (Fig. 6).

DISCUSSION

The three patients with carcinoma of the bronchus reported in this paper demonstrate the typical clinical syndrome of hypertrophic osteoarthropathy (HOA) characterized by peripheral edema relatively resistant to diuretic treatment, stiffness and pains in the extremities and bone tenderness with roentgenologically demonstrable periosteal proliferations. The explanation of the development of HOA is unknown. The opinions are divided between neurogenic and humoral mechanisms, though both could be active simultaneously (19)

The search for a humoral factor which could be responsible for the development of HOA has revealed that some patients with HOA and lung tumors had elevated urinary excretion of estrogens (15), and in some patients the urinary excretion of gonadotropic activity was elevated, but this was demonstrated too in some patients with lung tumor without HOA (9) In patients with the ectopic A.C.T.H. syndrome, cases have been reported with and without HOA (22, 25) Ive (20) has performed plasma HGH determination in one patient with metastatic carcinoma of the cervix uteri with acanthosis nigricans, bullous pemphigoid and HOA. In this patient the HGH level was low but the patient was on steroid treatment. Remtoft (24) estimated plasma HGH in a patient with idiopathic hypertrophic osteoarthropathy (pachy dermoperiostosis) and found normal values. Steiner et al. (26) described a patient with carcinoma of the bronchus, osteoarthropathy and elevated plasma HGH levels, which normalised after re section of the carcinoma. This patient had normal fasting blood sugar and plasma HGH determination were not performed during hypoglycemia or hyperglycemia. Using radioimmuno-assay and immunohistochemical techniques Cameron et al. (5) were able to demonstrate HGH in a bronchogenic carcinoma from a patient with HOA. The HGH concentration in the tumor was considerably higher than in the surrounding lung tissue. Plasma HGH was normal in this patient.

One of our patients (case 3) had elevated fasting plasma HGH concentration. There was no plasma HGH response to insulin-induced hypoglycemia or during the glucose tolerance test. The fasting blood sugar was elevated, the glucose tolerance test was diabetic, but there was elevated fasting plasma insulin level, with increase in the plasma insulin during hyperglycemia. This is in accordance with the findings in some acromegalic patients (3 7 11) although variations in HGH concentrations following decrease and increase in blood sugar concentrations in some acromegalic patients have been reported (6).

It is supposed that the lack of response to hypo- and hyperglycemia stimuli is due to autonomy of the hormone-producing tumor and/or disturbance of the feedback mechanism of the hypothalamic pituitary axis (1 3) In our patient the eye investigation and the X-ray of the sella turcica were normal, and clinically he was not acromegalic. The demonstration of elevated fasting plasma HGH levels and the autonomy of the HGH secretion could be explained by ectopic HGH production from the lung tumor

Finally it has been suggested by Greenwood et al. (14) as cited by Sterner et al. (26), that an elevated plasma HGH concentration in patients with breast cancer could be stress-induced. In these patients they frequently found a high plasma hydrocortisone level, as well as a significantly higher mean resting value of plasma HGH before and after mastectomy than in a healthy control population. Meanwhile the individual values were within the normal range.

In our patient the plasma corticosteroid level was normal, but the disturbances in the carbohydrate metabolism were identical with the steroid diabetes secondary to Cushing's syndrome (2).

In one of the patients (case 1) there was a subnormal increase in plasma HGH concentration following insulin-induced hypoglycemia before operation, while it was normal after removal of the tumor This might be due to an elevated plasma cortisol level (8, 27). In this patient it is tempting to postulate the presence of a tumor produced growth-hormone-like substance without the immunological activity of normal human growth hormone, but with biological activity This substance might be able to interfere with the central regulation of HGH secretion, which normalised after the operation.

The normal HGH secretion and fasting blood sugar in one of our patients with lung tumor and HOA indicate that this syndrome is probably not an ectopic HGH syndrome. But the findings of Steiner et al. (26) Cameron et al. (5) and the demonstration of autonomic and elevated HGH secretion in one of our patients indicate that cells from malignant lung tumors might produce HGH.

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DOSAGE OF HYDROXOCOBALAMIN FOR VITAMIN B_{12} DEFICIENCY

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Abstract. Serum B_{12} activity has been controlled frequently after one and/or two series of five i.m. injections of 1 mg hydroxocobalamin (OHB_{12}) on alternate days followed by injection of 1 mg OHB_{12} every three months for up to four years. Results from 23 patients with B_{12} deficiency indicated that one series of injections followed by four injections of 1 mg OHB_{12} a year is suitable for initial and maintenance therapy.

In a previous study (8) it was found that natural depots of B_{12} activity covering the needs of the body for 10–12 months were created by five intramuscular (i.m.) injections of 1 mg hydroxocobalamin (OHB_{12}) on alternate days to patients suffering from vitamin B_{12} deficiency. The daily demands were covered for at least three months by i.m. injection of 1 mg OHB_{12}, also during the early phase of restitution.

If reestablishment of normal conditions reduces considerably the consumption of B_{12} activity, the findings indicate that injection of 1 mg OHB_{12} every three months can cause abnormal extra- and intravascular concentrations of B_{12} activity dependent on the amounts stored during an initial depot treatment and the number of injections given three months apart as maintenance therapy.

For further elucidation of the dosage suitable for initial and maintenance therapy a study was therefore now made of how serum B_{12} activity varied during a period of up to three months in patients with vitamin B_{12} deficiency after five injections of 1 mg OHB_{12} on alternate days and after repetition of the procedure. Finally the activity patterns for time intervals between i.m. injections of 1 mg OHB_{12} three months apart were examined in patients first given an initial depot treatment of either one or two series of five injections of 1 mg OHB_{12} on alternate days.

METHODS AND MATERIAL

The investigation was based on determination of the B_{12} content of serum by microbiological assay using Lactobacillus leichmannii (2, 9). All measurements were performed in the Department of Biochemistry, Royal Dental College, Copenhagen, under the supervision of E. Hoff Jørgensen.

Twenty-three patients were included in the study. 19 patients had classical pernicious anemia, three patients had megaloblastic anemia following gastrectomy and one patient a non-megaloblastic anemia but values for serum B_{12} of 60–70 pg/ml.

Thirteen patients were untreated when included in the study. Ten patients were in hematologic remission following treatment; five of these were given only one injection of 1 mg OHB_{12} 3–6 months before the study, while one had another injection three months earlier. Four patients (Table I, cases 1, 6, 8, 10) were given a series of five injections of 1 mg OHB_{12} 11–13 months before the present study. One of these (case 6) also had a Schilling test one week before. However, all of the patients included in the study had no or only small B_{12} depots as estimated from values for serum B_{12} and/or the preceding therapy.

A commercial preparation of OHB_{12} was used throughout the study, containing 1 mg aqueous OHB_{12} in 1 ml stabilized, isotonic solution, pH 4.5 (Vibeden®).

RESULTS

The influence on serum B_{12} of repeated depot treatment with five i.m. injections of 1 mg OHB_{12} on alternate days and 2–3 months between the two series was examined in ten patients. Nine were hitherto untreated, while one (case 10, Table II, Fig. 3 *d*) was given 1 mg OHB_{12} 6 months before the first series of injections. As the serum B_{12} activity was measured every 1–5 weeks after both series, the patients served as their own controls.

After the initial series of five injections the serum B_{12} activity decreased in 3–6 weeks to

Table I. *Serum vitamin B three months after i.m. injection of 1 mg* OHB_{12} *given every three months as maintenance therapy after one initial series of five i.m. injections of 1 mg* OHB_{12} *on alternate days*

Case no	Sex	Body weight (kg)	No of injections	Serum vitamin B_{12} in pg/ml 3 mo after i.m. injection of 1 mg hydroxocobalamin: Min.	Max.	Average for 1st period of 1.5 y	Average for 2nd period of 1.5 y
1		6..	13	300	500	386	388
2		58	15	270	290	305	325
3		64	12	300	470	388	440
4		70	5	280	400	—	—
5		56	7	230	340	297	—
6		78	12	350	460	400	384
7		63	6	330	480	430	—
8		68	15	290	510	368	328
9	♂	79	1	180	500	200	431
10		64	15	270	370	334	320
11		68	12	330	420	358	374

values inside the normal range. For the first 3–4 weeks after the second series the concentration of B_{12} activity usually decreased as after the initial series, but later more slowly to a higher level than before (Figs. 1 and 3). In two patients (cases 2 and 10 Table II, Fig. 3 *a, d*) comparable serum concentrations were always higher after the second than after the first series.

The differences demonstrated for the two activity patterns obtained from the single subjects were never larger than the individual differences in the group obtained for the patterns after both the first and the second series of injections (Fig. 1). For the small group of patients examined no correlation was demonstrated between sex, age, body weight and starting values for serum B_{12} activity on the one hand, and the values obtained for serum B_{12} activity during up to three months after the injections on the other. Whether differences in depot treatment were of importance for the serum B_{12} concentration during a following maintenance therapy with i.m. injection of 1 mg OHB_{12} every three months was examined in the sequel.

Serum B_{12} *activity during maintenance therapy after one initial series of five i.m. injections of 1 mg* OHB_{12} was measured every 1–3 weeks in

Table II. *Serum vitamin* B_{12} *three months after i.m. injection of 1 mg OHB given every three months as maintenance therapy after two initial series of five i.m. injections of 1 mg* OHB_{12} *on alternate days and an interval of 1–3 months between series*

Case no	Sex	Body weight (kg)	No. of injections	Serum vitamin B_{12} in pg/ml 3 mo after injection of 1 mg hydroxocobalamin: Min.	Max.	Average for 1st period of 1.5 y
1	♂	45	10	440	1430	501
	♀	42	7	670	2200	1390
3	♀	54	7	300	690	430
4	♀	32	6	270	410	336
5	♀	65	9	490	2760	676
6	♂	62	8	420	650	520
7	♂	79	4	270	520	—
8	♂	67	7	420	800	564
9	♂	60	10	310	520	386
10	♂	65	11	540	1280	820
11	♂	70	9	260	390	363
12	♂	80	7	160	250	198

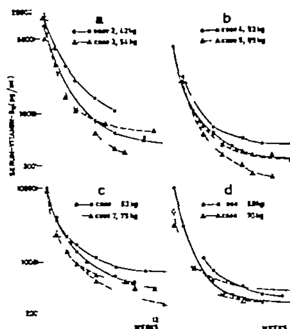


Fig. 1 (a–d) Serum vitamin B_{12} following five i.m. injections of 1 mg hydroxocobalamin on alternate days for B_{12} deficiency (O Δ), and following repetition of the same dosage 2 3 months later (●, ▲) in four female (*a, b*) and four male patients (*c, d*). For further details see text and Table II. Ordinate: log. scale.

11 patients. Sex, body weight, number of injections of 1 mg OHB_{12} and the range for serum B_{12} activity three months after injection are given in Table I. For eight patients controlled for three years or more the average values for the first and second period of 18 months are also given. All values were inside the normal range. For each patient the maximum differences measured were small the average values for the two consecutive periods of observation were practically uniform in seven of eight patients studied. The activity pattern between injections was uniform throughout the total period of observation in ten patients starting maintenance therapy 2–4 months after the initial treatment. This is illustrated by Fig. 2 *a* containing all values obtained for a patient controlled for four years. The high values for serum activity measured during the weeks after an injection were always lower than the corresponding values obtained after depot treatment. Later in the injection interval the values were still lower or of the same order as those obtained following the initial series of injections. All patients had values for B_{12} activity inside the normal range for 75% of the observation period or more. In the rest of the period higher values were obtained.

One patient started maintenance therapy five months after the initial series of injections (Table I, case 9). In the first part of the observation period the B_{12} activity decreased rapidly after an injection to values inside the lower part of the normal range. After 18 months of treatment the pattern changed. The activity decreased more slowly and 3–5 weeks after injection of 1 mg the activity level was higher than the comparable level obtained after the initial series. No further increase of the activity level between single injections was indicated from the values obtained after the following injections. The activity measured during the last two months of an interval was still inside the normal range for B_{12} activity of serum (Fig. 2 *b*).

Serum B_{12} activity during maintenance therapy after two initial series of five injections of 1 mg OHB_{12} 1–3 months apart was studied in 12 patients. Sex, body weight and the number of doses of 1 mg OHB_{12} injected at intervals of three months are given in Table II, also including values for the range of serum B_{12} obtained at the end of an interval and the average value obtained for the first 18 months of maintenance therapy It is seen that the range for B_{12} activity obtained in the patients was wider than found when maintenance therapy followed only one series of injections.

In seven patients (Table II, cases 3 4 6 7 9 11 12) the activity curve between injections obtained the highest level after the first injections given during maintenance therapy However in the major part of all intervals examined the values were inside the normal range. Later only small variations in activity were demonstrated from one interval to another

In five patients the activity level between injections increased from interval to interval. After a few injections most of the concentrations measured were higher than the corresponding values obtained after the second of the two initial series of five injections. In four patients the values were of the same order or higher than after depot treatment also during the first weeks after injection. After 3–9 injections all concentrations measured during an interval were above the normal

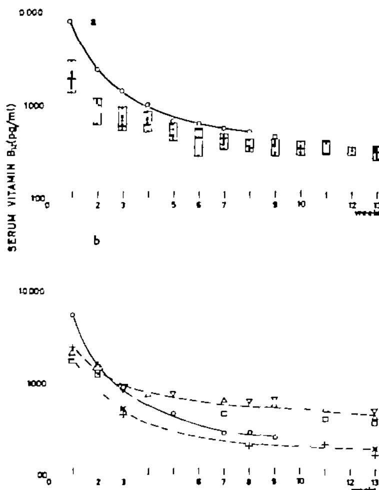


Fig 2. ( ) Serum vitamin B_{12} following five i.m. injections of 1 mg hydroxocobalamin on alternate days (O), and during maintenance therapy with i m. injection of 1 mg hydroxocobalamin every three months over four-year period. Minimum and maximum values obtained at uniform times after injection of 1 mg OH B_{12} given by bars including intervening values recorded during the study. (Case 10, Table I.)
(*b*) Serum vitamin B_{12} following five i.m. injections of 1 mg hydroxocobalamin on alternate days (O), and during maintenance therapy as in *a*, but started five months after the initial series of injections. The values obtained after 2, 6, 8 10 and 12 injections of 1 mg hydroxocobalamin are indicated (+ △ ▽ □) to demonstrate the shift from one activity course to another after 18 months of maintenance therapy (Case 9 Table I.) Ordinate: log. scale.

range (Fig. 3) Two of the five patients (Table II, cases 8 and 1) had the depot series with intervals of 1 and months, respectively and one patient (Table II, case 2) started maintenance therapy two months after the last depot series. The other intervals between injections were three months.

DISCUSSION

The high B_{12} concentration of serum after injection of OHB_{12} decreases in a few days to values not associated with renal excretion of B_{12} activity The rate of the further decrease depends on possibilities for intra- and extravascular binding, for distribution and metabolism of OHB_{12} and derivatives hereof. Under comparable experimental conditions an increase of serum B_{12} activity after repeated injection of OHB_{12} may be due partly to reduced extravascular capacities for binding, storage and consumption, partly to an increase of the intravascular binding capacity and/or hampered transfer of B_{12} products from the blood plasma to extravascular compartments.

In patients with untreated B_{12} deficiency given three or four injections of 1 mg OHB_{12} on alternate days, approximately 70% of the amount was retained in the organism (1 4) Thus, more than 3 mg was retained after five injections, or more than 75% of the calculated normal content of B_{12} vitamins (6). Repetition of this treatment caused variations in serum B_{12} activity not differing essentially for several weeks from those following the first series. Thus, the extravascular binding capacity for OHB_{12} surpassed the normal stores in the patients as previously found for normal individuals (8). Furthermore this activity response and the uniform pattern for serum B_{12} obtained for the intervals during maintenance therapy following one initial series of five injections, indicate that this treatment did not alter the

possibilities for OHB_{12} and products hereof to leave the intravascular compartment even at serum values inside the normal range. The shift of the activity level during maintenance therapy demonstrated in only one patient occurred after 18 months of treatment, which started later than in the other patients, therefore the new stable pattern was most probably caused by *reduction* of extravascular capacities for binding and/or storage of B_{12} activity. The final pattern was in the same range as in other patients, and at the end of each interval the serum concentration was in the middle of the normal range. Thus, after one initial series of five injections followed by four injections of 1 mg OHB_{12} a year the retention and metabolism of OHB_{12} matched in a way securing values for serum B_{12} activity inside the normal range for the last several weeks of each interval between injections.

The similar stable pattern obtained in seven out of 12 patients during maintenance therapy after two initial series of five injections indicates that a suitable ratio between retention and metabolism can be obtained also under such conditions; however the increasing activity level obtained in five other patients demonstrated clearly that this treatment often caused a supply surpassing the needs or increasing the intravascular binding of B_{12} products.

An argument in fa our of overloading is that the phenomenon never occurred in patients under identical maintenance therapy after only one initial depot series. Furthermore a short interval was used between the two depot series or before the start of maintenance therapy in three of the fi e patients concerned. Nothing indicated an action of abnormal plasma binding reported to be of importance for the high B_{12} content of serum after treatment with cyanocobalamin-tannate suspended in aluminum monostearate-sesame oil (Betolvex®) (3 5 7). As this last possibility could not be definitely settled from the present study it is now under investigation by other means. Whatever the reasons, the finding of an increase of the B_1 level in patients given two initial depot series indicates that in some patients one initial series of five injections of 1 mg OHB_{12} followed by four injections a year may also cause unnecessarily high serum values after several years of treatment. On the other hand larger intervals than three months between injections may cause under-

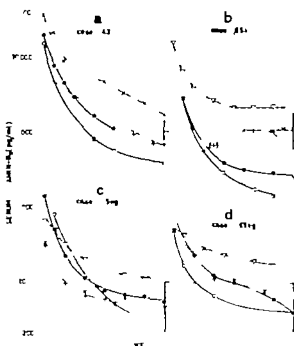


Fig. 3 (*a–d*) Serum vitamin B_{12} following five i.m. injections of 1 mg hydroxocobalamin on alternate days in two consecutive series with an interval of 3 months (O,●) to four patients with an increase of the serum vitamin B_{12} level during maintenance therapy with 1 mg hydroxocobalamin every three months. For all cases the values obtained after tw and four injections are given (+). For cases 5 (*b*), 1 (*c*), and 10 (*d*) values after 8 9 and 10 injections, respectively are also given. For further details see text and Table II. Ordinate: log. scale.

treatment in others. A close adjustment of dose intervals to the needs of the single patient can only be obtained under guidance of serum B_{12} measurements. Nothing indicates that surplus B_{12} activity does any harm, but one determination of serum B_{12} activity costs more than the amount of OHB_{12} used for maintenance therapy for four years using 4 mg OHB_{12} a year. Therefore it seems to be more safe and economic to use this dosage after an initial series of five injections and to control the serum B_{12} activity just before injection at intervals of years.

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IRON STORAGE IN PORPHYRIA CUTANEA TARDA

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Abstract. Iron stores have been quantitated by several methods in 30 patients with porphyria cutanea tarda (PCT). Twenty-seven had clinically manifest and three had latent disease. There were twenty-three men and seven women. The mean non-hemin iron concentration in liver biopsy specimens related either to dry weight or to protein content was significantly higher in PCT than in control male subjects. In patients with manifest PCT iron stores mobilizable for hemoglobin formation by frequent phlebotomy showed a mean value of 1.8 g and a highest value of 4.4 g. The desferrioxamine-induced urinary iron excretion was above the normal range for males in / of the patients with manifest PCT and the mean of the PCT group was significantly higher than that of the controls. All determinations of iron stores in porphyrica, however, showed wide variations between individuals and an appreciable overlap with controls. Although the size of the iron stores was definitely higher in the porphyric group, values within the range of idiopathic haemochromatosis were not encountered. Histochemical examination of the liver showed significantly greater amounts of stainable iron in parenchymal and in Kupffer cells in patients with PCT as compared with control subjects. All patients with manifest PCT had stainable iron in both parenchymal and Kupffer cells. Only a few in the control group had stainable iron in Kupffer cells. The porphyric group also had a higher amount of reticular iron in bone marrow sections. The mean serum iron concentration, the percentage saturation of transferrin and the percentage of sideroblasts were significantly higher in PCT as compared with the controls. The possible mechanisms which might be responsible for the increased iron stores in PCT are discussed.

Signs of disturbed iron metabolism have been reported in the symptomatic type of porphyria cutanea tarda (PCT). Thus, an elevated serum iron level is a common finding (5, 8, 14, 19, 20, 32, 33, 58) and the presence of increased amounts of histochemically visible iron in the liver has been reported (5, 13, 31, 37, 62). Radioiron turnover studies indicated iron overload in some cases (5, 56) but not in others (49). There are, however, no reliable data concerning direct measurements of the size of the iron stores. The estimation of histochemically visible iron in the liver does not give enough information on the range of storage iron encountered in this disease, since there is no close relationship between histochemically visible iron and iron determined chemically (43, 66). Furthermore such studies are meaningful only when compared with a control material and this has not previously been done.

The reports of a favourable effect of phlebotomy (20, 31, 32, 58) with subsequent depletion of iron stores suggest that there might be a causal relationship between the amount of storage iron and the clinical and biochemical activity of PCT which would also justify a precise quantitative study of the iron stores.

In the present study of 30 patients with PCT iron stores were determined by quantitative methods and compared with those of control subjects. In addition to estimation of histochemically stainable iron in the liver and bone marrow, iron concentration was determined chemically in liver biopsy specimens. The total available iron stores for hemoglobin synthesis were determined by frequent phlebotomy, and the chelatable stores were evaluated by the desferrioxamine (DF) test.

Some of the present results have been given in a preliminary report (41).

METHODS

The total storage iron available for hemoglobin synthesis was measured by frequent phlebotomy as described by Haskins et al. (27). Blood was removed by serial phlebotomies of 400–530 ml. At each phlebotomy the hemoglobin concentration was determined. Serum iron, total iron binding capacity and desferrioxamine-induced urinary iron excretion were determined before phle-

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Table I. *Sex, age estimated alcohol consumption and porphyrin excretion in the porphyric patients*

				Porphyrin excretion			
				Urine (mg/24 h)		Faeces (µg/g dry wt)	
Pat. no.	Sex	Age	Alcohol consumption	UP N <0.2	CP N <0.15	CP N <10	PP N <40
Manifest PCT							
1		57	Heavy	3.4	0.13	105	74
2	♂	45	Moderate	4.0	0.14	7	77
3	♂	63	Moderate	11.0	0.53	134	186
4	♂	74	Moderate	4.7	0.14	55	30
5	♂	47	Moderate	4.3	0.16	158	1[illegible]7
6	♂	50	Large	3.8	0.51	128	129
7	♂	50	Heavy	3.4	0.20	(5)[a]	(10)
8	♂	42	Large	2.8	0.36	28	7
9		57	Moderate	[illegible]4	0.25	8	15
10	♂	59	Heavy	6.6	0.38	(19)	(11)
11	♂	50	Minimal	6.3	0.34	(7)	(8)
1	♂	67	Heavy	3.4	0.[illegible]4	5	22
13	♂	54	Heavy	6.1	0.60	(9)	(7)
14	♂	53	Large	2.8	0.11	82	85
15	♂	49	Moderate	2.1	0.23	115	9
16	♂	7[illegible]	Large	5.4	0.[illegible]5	55	20
17	♂	58	Heavy	4.8	0.33	25	18
18	♂	44	Moderate	2.6	0.17	21	1[illegible]
19	♂	65	Moderate	5.8	0.24	40	17
20		63	Minimal	5.1	0.28	12	25
21	♂	47	Moderate	2.1	0.30	23	23
22	♀	69	Insignif.	8.2	0.31	10	14
23	♀	41	Minimal	5.8	0.18	53	64
24	♀	51	Large	3.7	0.19	5	6
25	♀	40	Small	3.5	0.46	80	73
26	♀	64	Insignif	4.7	0.38	352	311
27	♀	23	Large	3.6	0.13	78	83
Latent PCT							
28	♂	35	Large	1.2	0.10	24	48
29	♂	61	Heavy	0.9	0.08	5	8
30	♀	59	Insignif	0.3	0.10	9	45

[a] Values within brackets represent determinations performed after phlebotomy-induced remission.

urinary excretion of coproporphyrin (CP) usually was slightly or moderately increased, and so was faecal excretion of CP and protoporphyrin (PP). Faecal excretion of CP was more often increased than PP. In the three patients with clinically latent disease the urinary excretion of UP was slightly to moderately increased, while the excretion of CP in urine was normal. The faecal excretion of porphyrins in these patients was normal or slightly increased. The urinary excretion of porphyrin precursors (delta-aminolevulinic acid and porphobilinogen) was normal in all cases.

Microscopic examination of liver biopsy specimens (Table II) obtained from 24 patients showed varying degrees of siderosis (13 had more than 3% of visible fat), fibrosis (14 cases) and periportal round cell infiltration (16 cases). None had overt cirrhosis. In the woman with latent PCT (no. 30) thorotrast angiography was performed in 1943 and she had large amounts of brownish particles in the periportal areas.

Routine liver function tests (Table II) showed normal serum bilirubin levels in most patients, but BSPR was increased in 22 out of 25 and the SGOT and or SGPT levels in 23 out of 27 patients with manifest disease. Three patients with manifest PCT had overt diabetes. Intravenous glucose tolerance test in 22 other patients with manifest PCT revealed decreased glucose tolerance (Table II) in eight. The three patients with latent PCT had normal glucose tolerance.

None of the patients had received parenteral iron treatment or blood transfusions except for one male (no. 8) who was given two pints of blood four years before this study in connection with gastric resection for duodenal ulcer. No other had undergone gastric resection or had history of hemorrhage.

Control subjects

Normal values for hemoglobin, serum iron, total iron binding capacity and DF-induced urinary iron excretion

Table II. *Liver histology exocrine pancreatic function, glucose tolerance and liver function tests in patients with porphyria cutanea tarda*

	Liver histology			Pancreatic function		Liver function			
Pat. no.	Steatosis (per cent of visible fat)	Fibrosis	Periportal round cell infiltration	Trypsin (μg/ml)	Glucose tolerance (k-value) N >1.0	Bilirubin (mg/100 ml) N <1.1	GOT (U) N <40	GPT (U) N <40	BSPR (%) N <5
Manifest PCT									
1				240	0.95	0.3	33	41	10
2	5.0	Insignif.	Insignif.	775	2.35	0.6	26	58	7
3	11.5	Moderate	Moderate	310	Diabetes	0.7	51	69	9
4	7.8	Insignif.	Slight	485	1.31	0.5	41	50	14
5	6.8	Insignif.	Slight		0.75	0.5	37	61	16
6	3.5	Slight	Slight	360	0.83	1.4	60	55	19
7					1.07	1.4	85	80	25
8	1.3	Moderate	Marked		1.08	1.1	116	117	34
9	10.3	Insignif.	Insignif.		1.61	0.6	44	48	8
10	3.5	Moderate	Slight	240	1.63	0.6	72	101	20
11	10.4	Marked	Moderate	310	Diabetes	0.7	56	75	10
12	0.0	Marked	Moderate		1.39	0.7	115	162	32
13	2.1	Slight	Slight	380		0.4	58	50	
14	5.4	Insignif.	Insignif.		0.70	0.5	44	65	6
15	12.0	Slight	Slight		1.31	1.0	44	25	10
16	13.9	Insignif.	Insignif.		1.54	0.6	81	108	14
17	0.2	Slight	Slight	415	0.94	0.7	72	82	12
18	4.4	Slight	Slight	485	1.37	0.6	40	70	20
19	0.2	Slight	Slight	330	0.86	0.5	90	113	21
20					Diabetes	0.5	73	114	6
21	14.0	Insignif.	Insignif.	220	3.46	0.5	18	65	3
22	0.1	Insignif.	Slight	345	2.17	0.4	45	36	24
23						0.6	69	120	
24					1.78	0.4	56	71	2
25					0.64	1.1	72	87	3
26	0.3	Slight	Slight		0.82	0.3	40	24	10
27	0.0	Insignif.	Insignif.		1.51	0.5	60	80	14
Latent PCT									
28	0.2	Moderate	Slight		1.63	1.4	26	50	1
29	0.3	Insignif.	Insignif.	175	2.57	0.5	32	29	10
30	1.4	Slight	Insignif.		1.78	0.5	20	19	5

were obtained from 26 healthy male hospital employees and male medical students. The mean age was 38 years with range 21 to 60 years. In another control group consisting of 20 male patients who were admitted for cholecystectomy because of uncomplicated gallstone disease, histochemically visible iron and liver non-hemin iron concentration were determined in liver biopsy specimens. The mean age was 44 years with range 19 to 81 years. No patient in the above control groups had history of hemorrhage, and none had undergone gastric resection. They had never received blood transfusions or iron medication. These control series have been reported in detail elsewhere (42, 43).

The relationship of DF-induced iron excretion to liver iron concentration was studied in 14 males and nine females (five post-menopausal and four menstruating). One of them was admitted for operation of duodenal ulcer and the others for uncomplicated gall bladder disease. The normal range for sideroblasts in bone marrow smears and for stainable iron in bone marrow sections was obtained from 24 males in the surgical ward. The mean age was 46 years with range 23 to 62 years. They had no signs of infection.

Normal values for pancreatic function test according to the method of Lundh (38) were obtained from 15 healthy hospital employees and medical students. The mean age was 35 years with range 18 to 62 years.

RESULTS

Chemical determination of liver iron with dry weight and protein as reference (Tables III and IV Figs. 1 *a* and 1 *b*)

The mean non-hemin iron concentration with dry weight as reference base (Fig. 1 *a*) in 18 patients (16 males and two females) with manifest PCT (194 ± 40 mg/100 g) was significantly higher

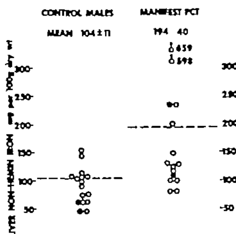


Fig. 1 a. Liver non-hemin iron with dry weight as reference in 20 control men and in 18 patients with manifest PCT ● omen. – mean values.

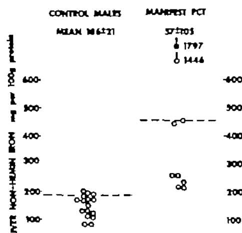


Fig. 1 b. Liver non-hemin iron with protein as reference. Symbols as in Fig. 1 *a*.

($p < 0.05$) than that of 20 control males (104 ± 11). With protein as reference (Fig. 1 *b*) the mean of the porphyrics with manifest disease (457 ± 105 mg/100 g) was also significantly higher ($p < 0.05$) than that of the controls (186 ± 21). As the liver iron concentration values showed a positive skew distribution and the logarithmic function of the iron concentrations provided a better approximation to a normal distribution, the logarithmic values were also analysed statistically. The mean log (iron concentration related to dry weight) of the 18 porphyrics was 2.186 ± 0.066 (corresponding to an iron concentration of 154 mg per 100 g dry weight) and that of the 20 control males was 1.976 ± 0.044 (corresponding to an iron concentration of 95 mg per 100 g dry weight). The difference between the means was significant ($p < 0.01$). The mean log (iron concentration related to protein) of the porphyrics was 2.539 ± 0.071 (corresponding to an iron concentration of 346 mg per 100 g protein) and that of the control group was 2.229 ± 0.041 (corresponding to an iron concentration of 169 mg per 100 g protein). The difference between the means was highly significant ($p < 0.001$).

The overlap between the iron concentration values of porphyrics and controls was less pronounced with protein as reference base. Two porphyrics had strikingly high iron concentration (1.8 and 1.4 g per 100 g protein).

Of the three patients with latent PCT one man had a high value (563 mg per 100 g protein) and the other a normal value (209 mg per 100 g protein). The woman had a low value (60 mg per 100 g protein).

There was no relation between the liver iron concentration and the amount of visible fat.

Mobilizable iron stores as determined by frequent phlebotomy (Tables III and IV Fig. 2)

Iron available for hemoglobin synthesis was estimated by means of frequent phlebotomy in 13

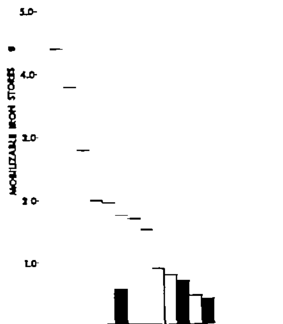


Fig. 2. Mobilizable iron stores in 13 patients with manifest PCT. Black bars, women.

Table II. *Liver histology exocrine pancreatic function, glucose tolerance and liver function tests in patients with porphyria cutanea tarda*

Pat. no.	Liver histology: Steatosis (per cent of visible fat)	Fibrosis	Periportal round cell infiltration	Pancreatic function: Trypsin (µg/ml)	Glucose tolerance (k-value) N.v > 1.0	Liver function: Bilirubin (mg/100 ml) N < 1.1	GOT (U) N < 40	GPT (U) N < 40	BSPR (%) N < 5
Manifest PCT									
1				240	0.95	0.3	33	41	10
2	5.0	Insignif	Insignif.	775	2.35	0.6	26	58	7
3	11.5	Moderate	Moderate	310	Diabetes	0.7	51	69	9
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5	6.8	Insignif	Slight		0.75	0.5	37	61	16
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8	1.3	Moderate	Marked		1.08	1.1	116	117	34
9	10.3	Insignif.	Insignif.		1.61	0.6	44	48	8
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13	2.1	Slight	Slight	380		0.4	58	90	
14	5.4	Insignif	Insignif		0.70	0.5	44	65	6
15	12.0	Slight	Slight		1.31	1.0	44	25	10
16	13.9	Insignif	Insignif		1.54	0.6	81	108	14
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21	14.0	Insignif	Insignif.	220	1.46	0.5	18	65	3
22	0.1	Insignif.	Slight	345	2.17	0.4	45	36	24
23						0.6	69	120	
24					1.78	0.4	56	71	2
25					0.64	1.1	72	87	3
26	0.3	Slight	Slight		0.82	0.3	40	24	10
27	0.0	Insignif.	Insignif		1.51	0.5	60	80	14
Latent PCT									
28	0.2	Moderate	Slight		1.63	1.4	26	50	1
29	0.3	Insignif	Insignif	175	2.57	0.5	32	29	10
30	1.4	Slight	Insignif.		1.78	0.5	20	19	5

were obtained from 26 healthy male hospital employees and male medical students. The mean age was 38 years with range 21 to 60 years. In another control group consisting of 20 male patients who were admitted for cholecystectomy because of uncomplicated gallstone disease, histochemically visible iron and liver non-hemin iron concentration were determined in liver biopsy specimens. The mean age was 44 years with range 19 to 81 years. No patient in the above control groups had a history of hemorrhage, and none had undergone gastric resection. They had never received blood transfusions or iron medication. These control series have been reported in detail elsewhere (42, 43).

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RESULTS

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The mean non-hemin iron concentration with dry weight as reference base (Fig. 1 *a*) in 18 patients (16 males and two females) with manifest PCT (194 ± 40 mg/100 g) was significantly higher

DF-induced iron excr in urine		Mobilizable iron	
Total (mg/24 h)	Per kg body wt (µg/24 h)	Total (g)	Per kg body wt (mg)
1 23	10 4		
1.07	13 0	1.54	18.8
2.59	32.0		
1 16	13 6	0 80	9 4
1.30	17.8	1 71	23.4
2.21	28 3	1.99	25.5
2.28	28.5	1.95	24 4
1 42	23.3	0 91	14.9
0 64	8 0		
4 64	57.3	3 78	46.7
4.33	61.9	4 42	63 1
1 10	16 9		
1 76	25 1		
0.73	9 4	0 46	5.9
1.30	14 0	2.79	30 0
1.32	15.5		
1.05	14 8		
1.62	16 2		
1.91	23.6		
2 94	37 7		
1 17	13.0		
0 99	13.2		
1.25	19.2		
1.07	16.0		
1.89	28.6	0 68	10 3
0 71	11.5	1 75	28.2
0 67	15 2	0 39	8 9
1.51	20.4		
0 60	10.2		
0.33	6.5	0 06	1.2

tion in mg per 24 hours are also given (Tables III and IV)

The relation between mobilizable iron stores and desferrioxamine-induced urinary iron excretion (Fig. 4)

The relation of DF-induced iron excretion (mg/24 h) to total mobilizable iron stores (g) was studied in 14 porphyrics. A correlation coefficient of 0.85 ($p < 0\ 001$) was obtained. The equation of linear regression of DF-induced iron excretion (mg/24 h) on mobilizable iron stores (g) was $y = 0.86x + 0.29$ The residual standard deviation was 0.7 mg at a mean DF-induced iron excretion value of 1 7 mg.

The relation between desferrioxamine-induced iron excretion and live iron concentration (Figs. 5 *a*, 5 *b* and 6)

The relation of DF-induced urinary iron excretion to liver iron concentration was studied in 23 controls (Table V Figs. 5 *a* and 5 *b*) and in 21 porphyrics (Table III Fig. 6). There was a statistically significant correlation both in the control group and in the porphyric group irrespective of which reference base was chosen (liver dry weight or liver protein content) (Table VI). The regression coefficients and the constants of the porphyric group did not differ significantly from those of the control group The residual standard deviations (R.S.D) of the porphyrics were however significantly higher than those of the controls.

Histochemical estimation of liver iron (Figs. 7 8 *a* and 8 *b*)

Histochemical estimation of liver iron was performed in 20 porphyrics with manifest disease (17 men and 3 women) and in 20 control men (Fig. 7) Porphyrics had significantly higher gradings of stainable iron in *parenchymal liver cells* than control men ($\chi^2 = 20\ 6$ D.F 4 $p < 0.001$). All patients with manifest PCT studied had stainable iron of grade 2 + or more and two had stainable iron of the highest grade. Of 0 control men, seven had grade 2 + or 3 + No control had grade 4 +

The difference between porphyrics with manifest disease and controls was still more pronounced with respect to stainable iron in *histiocytic cells* ($\chi^2 = 32.4$ D.F 4 $p < 0\ 001$). Iron in histiocytic cells was found in only three out of 20 male controls (two had grade 1 + and one had grade 2 +), but 18 out of 20 patients with manifest PCT had grade 2 + or 3 +

The relationship between histochemically visible iron in liver parenchymal cells and iron determined chemically was studied in 20 male controls and in 21 porphyrics (Figs. 8 *a* and 8 *b*). Except for grade 0-trace there was a wide range of iron concentration values in the different histochemical gradings and an appreciable degree of overlap With dry weight (Fig. 8) as reference the chemical iron values of porphyrics with grade 3 + of histochemical iron showed a considerable overlap with the values of controls with histochemical iron of grade 1 + With protein as reference (Fig. 8 *b*) there was no such overlap

Table IV *Results of iron studies in patients with manifest porphyria and in control males Means, S.D., S.E. of mean, ranges, and analysis of the differences between the means of porphyrics and controls*

	Manifest PCT						Control males				
	Sex		Mean	Range	S.D	S.E. of mean		Mean	Range	S.D	S.E. of mean
Hemoglobin (g/100 ml)	♂	21	14.6	12.9–16.7	0.9	0.2	26	14.6	13.5–16.4	0.8	0.1
	♀	6	13.3	12.8–13.6	0.3	0.1					
Serum iron (μg/100 ml)	♂	21	193	129–300	47	10	26	131	72–225	38	7
	♀	6	159	114–206	39	16					
	♂+♀	27	186	114–300	47	9					
TIBC (μg/100 ml)	♂+♀	27	314	198–424	54	10	26	331	264–417	44	9
TIBC saturation (%)	♂	21	60	36–91	15	3	26	41	25–79	13	3
	♀	6	60	44–80	15	6					
	♂+♀	27	60	36–91	14	3					
Sideroblasts (%)	♂	17	78	61–90	8	2	21	55	16–86	23	5
	♀	6	65	45–75	10	4					
	♂+♀	23	74	45–90	10	2					
DF-induced iron excr (mg/24 h)	♂	21	1.80	0.64–4.64	1.07	0.23	26	0.78	0.47–1.35	0.22	0.04
	♀	6	1.10	0.67–1.89	0.45	0.18					
	♂+♀	27	1.64	0.64–4.64	1.00	0.19					
DF-induced iron excr (μg/kg body wt/24 h)	♂	21	23.3	8.0–61.9	14.3	3.1	26	10.1	6.9–14.9	2.1	0.4
	♀	6	17.3	11.5–28.6	6.1	2.5					
	♂+♀	27	21.9	8.0–61.9	13.1	2.5					
Liver iron (mg/100 g dry wt)	♂+♀	18	194	62–659	168	40	20	104	45–247	50	11
Liver iron (mg/100 g prot.)	♂+♀	18	457	123–1797	445	105	20	186	85–485	92	21
Mobilizable iron (g)	♂	10	2.0	0.5–4.4	1.3	0.4					
	♀	3	0.9	0.4–1.8							
	♂+♀	13	1.8	0.4–4.4	1.2	0.3					

Different variances. Adjustment of the degree of freedom according to Welch (67).

Stainable iron in bone marrow sections and sideroblast counts in bone marrow smears (Tables III and IV Fig. 9)

Fig. 9 shows that porphyrics had more stainable iron in bone marrow sections than male controls. Seventeen out of 20 porphyrics with manifest disease had grade 2+–4+ but only five out of 18 male controls had stainable iron of grade 2+ or more ($\chi^2 = 12.7$ $p < 0.001$)

The mean sideroblast count in 23 porphyrics (17 men and 6 women) was 74 ± 2 %. This was significantly higher than the mean value in a control series comprising 21 hematologically normal males (55 ± 5 %).

Serum iron, total iron binding capacity and TIBC saturation (Tables III and IV Figs. 10 and 11)

The mean serum iron concentration of the 27 patients with manifest PCT (186 ± 9 μg per 100 ml) was significantly higher ($p < 0.001$) than that of the normal males (131 ± 7 μg). Eleven porphyrics had serum iron values above 200 μg per 100 ml (Fig. 10)

The mean TIBC value of the porphyric patients (314 ± 10 μg per 100 ml) was lower but not significantly different from that of the control males (331 ± 9) Three porphyrics with manifest disease had values below the range of the control males.

The mean TIBC saturation value of patients with manifest PCT (60 ± 3 %) was significantly higher than that of the male control group (41 ± 3 %). On the other hand most porphyrics had values within the range of the control males (Fig. 11)

Pancreatic exocrine function (Table II)

The pancreatic function test was performed in nine porphyrics before phlebotomy treatment. The

t-test of mean values (PCT vs. control males)		
t	D.F	*P*
0		
5.0	45	<0.001
1.6	30	<0.20
4.6	51	<0.001
1.2	51	<0.30
4.7	45	<0.001
3.2	30	<0.005
5.0	51	<0.001
4.3	25[a]	<0.001
1.5	19[a]	<0.20
3.6	27[a]	<0.005
4.3	21	<0.001
1.7	6[a]	<0.20
4.4	29[a]	<0.001
4.2	21	<0.001
2.8	5[a]	<0.05
4.6	27[a]	<0.001
2.2	20[a]	<0.05
2.5	18[a]	<0.05

mean trypsin value was 374 ± 60 with a range of 175 to 775 μg per ml. In five others the test was performed after phlebotomy. Their absolute mean value was slightly lower (341 ± 41 with range 240 to 485), but not significantly different from those in which the test was performed before treatment. The mean trypsin value of all porphyrics studied (362 ± 40) was not significantly different ($p > 0.50$) from the mean value of 15 controls (401 ± 45). No porphyric had a trypsin value below the range of controls (150 to 710 μg per ml duodenal content).

DISCUSSION

According to several authors iron metabolism is altered in PCT. The most frequent finding was an increased serum iron level (5 8 14 19 20 32, 33 58) and the presence of stainable iron in the

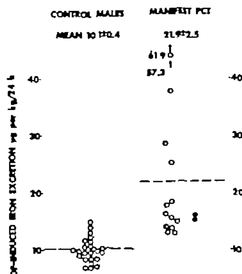


Fig. 3. Desferrioxamine-induced urinary iron excretion (μg per kg body weight per 24 h) in 26 male controls and in 27 porphyrics with manifest disease. Symbols as in Fig. 1 a.

parenchymal liver cells (5 13 31 6..). The latter has been regarded as a sign of siderosis. An increased serum iron level is, however, a common finding in active liver disease without iron overload, and though massive crude clumps of histochemically visible iron in parenchymal liver cells

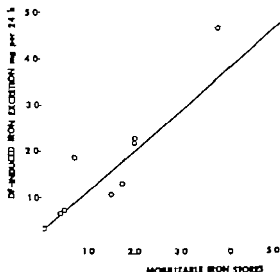


Fig. 4. The relationship between mobilizable iron stores and DF-induced urinary iron excretion in 14 porphyrics. $y = 0.86x + 0.29$, $r = 0.85$, $t = 5.6$, $p < 0.001$

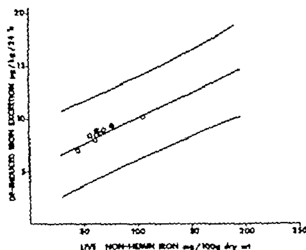


Fig. 5a. Relation of DF-induced iron excretion to liver non-hemin iron concentration with dry weight as reference of 23 controls. Regression line and 95% confidence limits are depicted. $y = 0.048x + 5.2$, $r = 0.75$, $t = 5.2$, $p < 0.001$

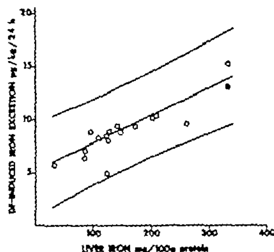


Fig. 5b. Relation of DF-induced iron excretion to liver non-hemin iron concentration with protein as reference of 23 controls. Regression line and 95% confidence limits are depicted. $y = 0.026x + 5.2$, $r = 0.73$, $t = 5.0$, $p < 0.001$

are related to an increased liver iron concentration, a moderate amount of stainable iron is a frequent finding in hematologically normal males (43, 66).

More accurate data on the quantity of iron stored in PCT are not available in the literature. Chemical analysis of liver iron in PCT has previously been performed in single cases. Thus Brugsch et al (12) in one case found a total liver iron of 670 mg which was considered to be normal. Berlin and Brante (6) analysed liver non-hemin iron in three cases with PCT and obtained values of 56, 92 and 13_ mg per 100 g dry weight (recalculated from wet weight). Those values are within the range of our male controls (45–247 mg per 100 g dry weight). In the present study different methods for the quantification of iron stores were used and compared with a control material. The mean liver non-hemin iron concentration calculated on dry weight and on liver protein as a reference in 18 porphyrics with manifest disease was found to be significantly higher

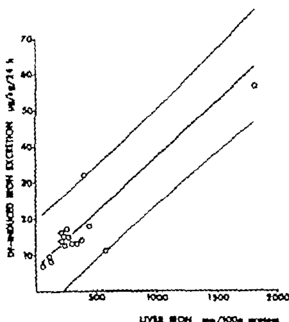


Fig. 6. Relation of DF-induced iron excretion to liver non-hemin iron concentration with protein as reference of 21 porphyrics. Regression line and 95% confidence limits are depicted. $y = 0.031x + 6.$, $r = 0.92$, $t = 9.9$, $p < 0.001$.

PARENCHYMAL CELLS | HISTIOCYTIC CELLS

CONTROL MALES | MANIFEST PCT | CONTROL MALES | MANIFEST PCT

STAINABLE LIVER IRON grade 0–4

Fig. 7. Histochemical iron in parenchymal liver cells (left) and in histiocytic liver cells (right) of 20 male controls and of 20 porphyrics with manifest disease. ● = omen

Table V *Relation of desferrioxamine-induced urinary iron excretion to liver iron in controls*

Subject no.	Age	Hb (g .)	Fe/s (μg)	TIBC (μg)	TIBC Satur (%)	Liver iron Fe/dry wt (mg 100 g)	Liver iron Fe/prot. (mg/100 g)	DF-induced iron excr in urine Total (mg/24 h)	DF-induced iron excr in urine Per kg body (μg/24 h)
Men									
1	48	15 4	192			92	172	1 16	14.5
2	30	13.5	81	314	26	153	258	0 58	9 5
3	44	14 6	160	366	44	144	332	1 12	13 0
4	21	13.6	138	339	41	99	173	0.57	8.5
5	35	14 8	126	397	32	192	333	0 97	15.2
6	81	13 9	36	365	10	103	183	0.59	7 2
7	49	14 1	145	378	38	105	203	0.74	10.1
8	63	14.0	137	350	39	61	125	0 61	8 1
9	65	14.1	95	281	34	61	111	0 49	8.2
10	40	14 5	151	400	38	143	233	1 24	14 4
11	67	13 6	177	345	51	103	172	0.76	9.3
12	19	15 1	79	400	20	45	86	0 69	7 0
13	50	14 8	149	304	49	122	207	0 95	10 3
14	40	14 8	91	368	25	76	142	0 98	9 3
Non-menstruating women									
15	62	12.9	134	333	40	56	96	0.56	8.4
16	55	14 6	128	305	42	68	147	0.70	8 8
17	62	12.6	69	337	20	62	126	0.49	8.6
18	55	13 4	114	259	44	63	125	0 63	8.4
19	66	12 9				57	123	0.40	4.9
Menstruating women									
20	42	12.8	80	293	27	41	65	0.42	8 9
21	36	13 1	113	360	31	51	85	0.42	6.3
22	46	12 6	88	342	26	18	34	0 42	5 7
23	44	12 8	183	375	49	70	116	0 71	10.4

than that of 20 control men. Two had strikingly high values, though not in the range of fully developed idiopathic hemochromatosis. With protein as reference base all but two had values above the mean value of controls. There was, however considerable overlap (Fig. 1 *b*). With dry weight as reference the degree of overlap was still greater (Fig. 1). However since many cases f PCT had an appreciable degree of steatosis, results obtained with protein as reference are probably more relevant. Steatosis implies dilution of liver constituents with fat and increased dry weight because of the low water content of fat, thus giving lower values for iron concentration if dry weight is used as a reference (43).

There was no evidence of any causal relation

Table VI. *The relation between DF-induced iron excretion and liver iron concentration in controls and porphyrics*

Group		DF-induced iron excretion (*y*) Mean ± S D. (μg/kg bw)	Liver iron (*x*) Mean ± S D	Regression equation	R S D			*p*
			Mg/100 g dry wt					
Controls	23	9.3 ± 2 7	86 ± 42	*y* 0 0482*x* + 5 19	1 83	0 750	5 22	0 001
PCT	21	19 7 ± 14.5	184 ± 161	*y* 0 0825 4 53	6 00	0 916	9.92	0 001
			Mg/100 g protein					
Controls	23	9.3 ± 2.7	199 ± 77	*y* 0 0259*x* 5 24	1 88	0 734	4 96	0 001
PCT	21	19 7 ± 14.5	431 ± 434	*y* − 0 0314*x* 6 15	6 00	0 916	9.90	0 001

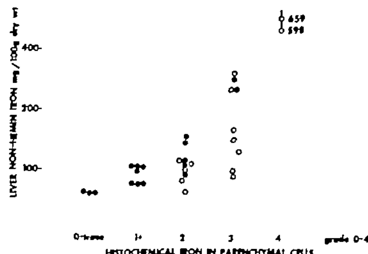


Fig 8 a. Relation of liver non-hemin iron concentration with dry weight as reference base to the grade of histochemical iron in liver parenchymal cells. ● controls, O porphyrics.

ship between increased liver iron concentration and structural liver changes. The two patients with the highest iron concentrations had marked or moderate degrees of fibrosis, but the same degree of fibrosis was also present in porphyrics with iron concentrations within the range of controls.

Determination of the total iron stores available for hemoglobin formation by means of frequent phlebotomy showed a wide range of values (0 4 to 4 4 g) with a mean of 1.8 g. Seven out of 13 patients had values above 1.5 g. According to available reports from the literature mobilizable

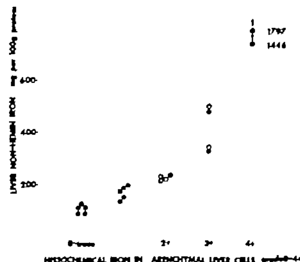


Fig 8 b Relation of liver non-hemin iron concentration with protein as reference to the grade of histochemical iron in liver parenchymal cells. ● controls; O porphyrics.

iron stores of more than 1.5 g are uncommon in normal males. Thus Haskins et al (27) mobilized an average of 0 84 g in two normal males, and Pritchard and Mason (50) mobilized 0.58 0 94 and 0 94 g in three normal males. Balcerzak et al (4) reported values of 0 13 to 1 90 with a mean of 0.56 g in 11 hematologically normal males. Only one had a value above 1 g. In the present study ten male porphyrics had a mean value of 2.0 g, and six of them had values above 1.5 g. Of three porphyric females with manifest disease one nonmenstruating (no. 46) had definitely increased stores (1 8 g) No porphyric with manifest disease had a value below 0 4 but the woman with latent disease had less than 0 1 g of storage iron.

The desferrioxamine test which has been reported to be valuable in the quantification of iron stores (4 24 25 48) showed increased urinary iron excretion in most of our patients with manifest PCT twenty out of twenty-seven patients had a DF induced iron excretion above the range of the normal males. Similar results were reported by Schnack and Wewalka (59) The DF test must, however be interpreted with caution in cases of active liver disease Schnack and Wewalka found increased DF-induced iron excretion during the acute phase of infectious hepatitis. We made the same observation in alcoholic liver disease (42) the DF-induced iron excretion was greater in alcoholics shortly after admission, when the serum transaminase levels were increased, than some week or so later when the transaminase levels had decreased Furthermore, in controls we found a

highly significant correlation between the DF induced iron excretion and the non-hemm iron concentration in liver biopsy specimens, but in alcoholics, most of whom had increased transaminase levels, the correlation was not significant. In the present porphyric series there was, however a highly significant correlation between the DF-induced iron excretion on the one hand and mobilizable iron stores (Fig. 4) or the liver iron concentration on the other (Fig. 6) Thus the DF test appears to mirror the size of the iron stores in PCT In two porphyrics the DF-induced iron excretion was considerably higher than predicted from the size of mobilizable iron stores or from their liver iron concentration (nos. 3 and 25). These had increased serum transaminase levels. It is possible that the increased DF-induced iron excretion values in the above patients were due to active liver disease. In one patient several DF tests gave normal iron excretion in spite of increased liver iron concentration and increased mobilizable iron stores (no 26). The cause of this decreased chelatability is obscure.

Thus determination of liver iron concentration, mobilizable iron stores and DF-induced iron excretion showed that porphyrics as a group had increased iron stores but with a wide range of individual values and an overlap with controls. No patient with manifest disease had decreased iron stores, and none had values within the range of fully developed idiopathic hemochromatosis.

Histochemically demonstrable liver iron was present in all of the PCT patients in the present series. No patient with manifest disease had stainable iron in parenchymal cells of less than grade 2+ and two had amounts suggestive of hemochromatosis. One male with latent PCT had also the highest grade of stainable iron in parenchymal cells. In the control series histochemical liver iron was demonstrated in most males, and similar results were obtained in a previous study of hematologically normal men (66). Nevertheless, significantly more patients with manifest PCT had higher gradings than the male controls. Also in other studies of PCT histochemically demonstrable iron in parenchymal liver cells was a consistent finding (5 13 31 6..). It has been suggested (5) that

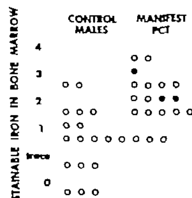


Fig 9 Histochemical iron in bone marrow sections of 18 control men and of 20 porphyrics with manifest disease. ● women.

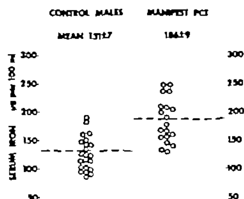


Fig 10 Serum iron in 6 normal males and in 27 patients with manifest PCT ● women.

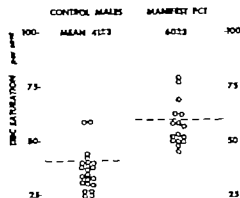


Fig 11 Transferrin saturation in 26 normal males and in 27 porphyrics with manifest disease. ● women.

an increase of hemosiderin at the expense of ferritin without increase of total iron in the liver cells might be the explanation of the increased amounts of histochemical liver iron in PCT. The relationship between histochemical iron in parenchymal liver cells and liver non-hemin iron determined chemically with dry weight as reference (Fig. 8 *a*) might support this suggestion, but most porphyrics had steatosis, which results in lower iron values if dry weight is used as reference (43). With protein as a base (Fig. 8 *b*) for the chemical iron concentration the relationship between histochemical iron in parenchymal liver cells and chemical liver iron in porphyrics was similar to that of controls.

Concerning iron in Kupffer cells the difference as compared to controls was still more pronounced. All porphyrics with active disease had stainable iron in Kupffer cells, while it was present in only three out of twenty control men. In some patients with PCT there was an abundant amount of histochemical iron in Kupffer cells in spite of a moderate amount of histochemical iron in parenchymal cells, which was never the case in controls. Our histochemical findings are in agreement with the results obtained by other authors (13, 62) who also emphasized the increased amounts of Kupffer cell iron. Disproportionate amounts of hemosiderin in histiocytic cells in the liver as compared with that in parenchymal cells was thought to be due to necrosis of parenchymal liver cells with release of iron pigment being phagocytosed by histiocytes (62). Liver cell necrosis was not a histopathological feature in the present series, but other signs of liver damage (steatosis and fibrosis) were common and the serum transaminases were increased in most cases.

Hemosiderin in bone marrow sections was also increased in the porphyric group as compared with the control group. High grades of reticuloendothelial iron were also present in a few patients with normal liver iron concentration (nos. 16 and 29) or a normal size of mobilizable iron stores (no. 27).

Increased reticuloendothelial deposits of hemosiderin may occur after parenteral iron administration, blood transfusions and in many hematological disorders, especially those with hemolysis. Increased in-vitro photo-hemolysis in PCT has been reported (35). Berman et al. (7) found normal erythrocyte survival in eight patients with PCT but in a study by Price et al. (49) the red cell life span was decreased (55–83 days) in three out of six patients. In the present series no porphyric had been treated with parenteral iron or blood transfusions. None had overt anemia. Slightly increased reticulocyte counts and decreased haptoglobin levels occurred, however, in single patients. It is possible that some patients had an increased hemolysis. Recurrent liver cell necrosis with release of ferritin from liver cells which is then phagocytosed by histiocytes in the liver and bone marrow might be another possible cause of increased reticuloendothelial iron. Ferritin in serum has been demonstrated in experimental liver cell damage (51) and in human hepatocellular disease (52).

Increased serum iron levels which were reported in PCT (5, 8, 14, 19, 20, 32, 33, 58) were also found in the present study. The mean serum iron concentration of the porphyrics was significantly higher than that of normal males. High serum iron levels were also present in three patients with normal mobilizable iron stores (nos. 9, 25 and 27). The reason for this is not clear but liver injury might have contributed to a high serum iron level. As in other studies (5, 19), high TIBC saturation values were common in the porphyrics. In fully developed hemochromatosis serum iron usually exceeds 200 µg% and TIBC saturation is usually above 90% (9). In patients with PCT the degree of transferrin saturation, although significantly higher than in normals, was not as high as in hemochromatosis. The sideroblast count was higher in porphyrics than in controls and corresponded to the high serum iron level. Although there is no correlation between the sideroblast count and serum iron in normal subjects (65) their number is usually increased in hemolytic states and anemias with high serum iron levels (3, 26).

The mechanism responsible for increased iron stores in PCT is not clear. Theoretically, a positive iron balance may be accomplished either by increased absorption or decreased excretion. Since the observations of McCance and Widdowson (44) it has been generally accepted that iron balance in man is mainly dependent on variations in absorption. Hitherto no case of positive iron balance due to decreased excretion has been demonstrated. On the contrary, increased iron excretion has been shown in populations with increased iron stores

(23). Increased iron uptake from the gastro-intestinal tract may be caused either by an increased alimentary load of iron, which is considered to be the main cause of iron overload in the South African Bantu population (11) or by an increased absorption rate from ordinary food iron. Excessive intake of alcohol has been proposed as a possible cause of increased iron absorption in PCT (19 37 57). Alcoholic beverages brewed in iron containers in South Africa (10) and some wines in France and Italy (1 47) contain large quantities of iron. Furthermore, radio-iron studies showed increased absorption of ferric iron if alcohol was added to the test dose (15) A high intake of alcohol might thus contribute to increased iron absorption even if the alcoholic beverage does not contain significant amounts of iron.

The diet and the cooking habits of the present porphyric group were not found to differ from those of the ordinary Swedish population. Their alcohol consumption consisted mainly of distilled spirits which do not contain significant amounts of iron. Analysis of cheap wines in Göteborg showed a mean iron content of 10 mg per l (43) Only two patients (nos. 8 and 12) consumed significant amounts of wine. In a previous study of a group of alcohol abusers we found rather a lower iron concentration in the liver as compared with the present control group (43) Furthermore, increased iron stores were found also in patients who did not consume significant amounts of alcohol (nos. 11 and 26) Thus, it is not probable that either dietary iron overload or alcohol contributed to the increased iron stores in the PCT patients in the present series.

Alcoholic steatosis has been reported to be associated with an increase of stainable iron in the liver (18), and increased radio-iron absorption was found in choline deficiency induced steatosis of the rat (46). However in a previous study of alcoholics in Göteborg liver iron was not increased in alcoholic steatosis (43) and in the present study there was no significant relationship between liver iron and the amount of visible fat. Increased absorption of radio-iron has also been reported in cirrhosis of the liver (17 68) and in chronic pancreatitis (16, 17). No porphyric had overt cirrhosis and none had clinical signs of pancreatic insufficiency In addition, all porphyrics studied with the pancreatic function test according to Lundh (38) had trypsin values within the normal range.

Thus the study did not indicate that increased iron stores in our PCT patients were related to cirrhosis of the liver steatosis or decreased exocrine function of the pancreas.

A basic question is whether the storage iron may influence the porphyrin metabolism in this disease The results showed that most patients with PCT had increased iron stores. On the other hand manifest PCT occurred also in patients with normal iron stores. Preliminary studies (41) have shown that exhaustion of iron stores by phlebotomy was consistently associated with loss of active porphyric skin symptoms and a markedly diminished porphyrin excretion, irrespective of the size of the original iron stores. However in two patients who were given iron in conjunction with phlebotomy treatment the uroporphyrin excretion remained at a high level. Iron administered after completion of the phlebotomy treatment was followed by relapses, but no relapse was seen in the other patients who were not given iron. It is noteworthy that the only patient with low iron stores (no 30) had latent PCT On the other hand in two other patients (nos. 28 and 29) the disease was inactive and the uroporphyrin excretion was low in spite of ample or increased iron stores. Thus there must be factors other than the size of iron stores that determine whether or not this disease becomes manifest. However it is probable that exhaustion of iron stores has a favourable effect on the biochemical and clinical manifestations of PCT (41)

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THE LIPEMIA IN FAMILIAL PLASMA LECITHIN CHOLESTEROL ACYLTRANSFERASE DEFICIENCY

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Abstract The earlier investigations of female patient with familial plasma lecithin cholesterol acyltransferase (LCAT) deficiency showed that a good deal of the triglyceride-rich lipoproteins had physical properties similar to those of the chylomicrons. Moreover the heparin-induced lipoprotein lipase activity (LLA) was distinctly reduced. The present study had the purpose of determining the type of lipemia in LCAT deficiency For eek the patient had high fat diet, the next week high carbohydrate diet. The lipemia was carbohydrate-induced. The top fraction on the polyvinylpyrrolidone (PVP) density gradient strongly increased when the patient was given diet extremely rich in carbohydrate. A broad beta-lipoprotein band followed by marked trail could be seen by lipid electrophoresis. There were very small amounts of alpha lipoprotein during the whole experiment. The results are discussed, particularly with regard to the possible effect of lack of LCAT activity on the plasma lipoproteins.

A new inborn error of metabolism, where the basic defect is probably the decreased activity of plasma lecithin cholesterol acyltransferase (LCAT), has recently been reported from Norway (7 15 16, 17). Clinical key symptoms are corneal opacity albuminuria and anemia with a slight hemolytic component.

The results of the lack of enzyme activity are typical alterations of the plasma lipids. The esterified part of the cholesterol is always very low the concentration of lecithin is high and that of lysolecithin low Total phospholipids, cholesterol and triglycerides are most often elevated.

A second family with the disease has been found in Sweden (9) A man god 41 died of uremia, most probably caused by the disease. His sister was examined in greater detail. Her post absorptive plasma was lipemic. In agreement with earlier findings using other techniques (electrophoresis, immunoelectrophoresis, precipitation) the ultracentrifugal analysis also showed a very small concentration of alpha-lipoprotein. A decreased cholesterol/phospholipid ratio in the very low density (VLD) and particularly in the low density (LD) lipoproteins was another notable finding. The separation on the PVP density gradient revealed that about 23% of the triglycerides were located in the top fraction. The heparin-induced LLA was distinctly reduced (9)

The low heparin-induced LLA in combination with high triglycerids in the top fraction on the PVP density gradient is in agreement with the findings in f t-induced lipemia (hyperlipoproteinemia type I according to Fredrickson et al. s nomenclature (6)) The clinical symptoms of LCAT deficiency and fat-induced lipemia are quite different, however

In order to determine the type of lipemia in LCAT deficiency the following study was performed.

METHODS

The examined patient was M. L., the Swedish female patient with LCAT deficiency hose clinical and laboratory dates have been published elsewhere (9). The patient was hospitalized during the whole study At first she received the ordinary hospital food for some days. Then, for about a week, she had high fat diet, followed by period with high carbohydrate diet. Three experiments were performed. Two of them, in the autumn of 1967 and summer of 1969 (*A* and *B*), were almost identical The high fat diet contained 140–155 g fat and 50–60 g carbohydrate, while the high carbohydrate diet was composed of 40–50 g fat and 325–350 g carbohydrate. The contents of protein and the caloric intake were kept constant: 110–115 g and 2100–2400 kcal respectively In the third study in the spring of 1969 (*C*) it was impossible to keep the mentioned diets on account f the patient' dyspeptic discomfort. This

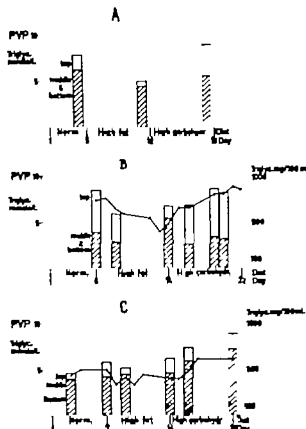


Fig 1 Variations in plasma triglyceride concentration during three different dietary experiments (*A*, *B* and *C* cf. text). The columns indicate the results in mmole/l on the PVP density gradient, where the hollow parts represent the top fractions. Plotted points, the total triglyceride values in mg 100 ml according to the method f Laurell (12)

time the fat and carbohydrate contents in the high-fat week were 125–145 g and 50–60 g, respectively while the corresponding values for the high-carbohydrate week were 40–55 g and 220–300 g The protein content varied from 100 to 125 g and the caloric intake from 1750 to 2000 kcal.

The lipoproteins in plasma ere determined in the postabsorptive state. They were separated by paper electrophoresis according to the method of Lees and Hatch (13) and were stained with Oil Red O according to Jencks and Durrum (10). They were also separated by agaros gel electrophoresis according to the method of Johansson (11), and were stained ith Sudan Black B. (The analyses by agaros gel electrophoresis were performed by Dr Bengt Johansson, Central Laboratory of Clinical Chemistry Allmänna sjukhuset, Malmö.) Furthermore, the lipoproteins were measured with the preparative ultracentrifuge according to Bragdon et al. (3) and their contents of major lipids were quantitatively measured. To separate the triglyceride-rich lipoproteins the polyvinylpyrrolidone (PVP) density gradient method according to Hallberg (8) was used, and the triglycerides were estimated according to Carlson (5). (The analyses in the preparative ultracentrifuge and on the PVP density gradient were performed by Dr Jonas Boberg, King Gustaf V Research Institute, Karolinska sjukhuset, Stockholm) Alpha-lipoproteins were also measured after precipitation f the beta-lipoproteins with manganese chloride and heparin according to Burstein and Samaille (4). Total and esterified cholesterol and total triglycerides were followed according to the methods of Babson et al. (1) and Laurell (1), respectively

RESULTS

The finding by separation on the PVP density gradient, mentioned above, suggested that part of the VLD lipoproteins had physical character istics common with chylomicrons, the top frac tion being the known place for the exogenous triglycerides. Other findings supported the suspicion that the patient had unusual triglyceride-rich lipoproteins. At lipoprotein electrophoresis there was a broad beta band followed by a marked trail No distinct pre-beta band was seen. When plasma was strongly lipemic, the triglyc erides could not be determined by estimating the light scattering intensity because this method gave extremely high values, probably due to particles, similar to chylomicrons.

All three studies showed that a high carbohydrate diet caused increasing lipemia. Moreover experiments *A* and *B* with a higher carbohydrate diet and higher caloric intake gave a very distinct increase of the top fraction on the PVP density gradient. This was not the fact in experiment *C* The results of the three studies with the varia tions of plasma triglycerides and the top frac tion on the PVP density gradient are shown in Fig. 1

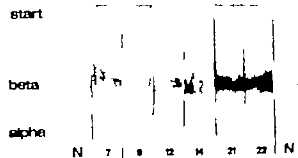


Fig 2 Paper electrophoretograms of the plasma lipoproteins during the dietary study *B* For comparison two normal electrophoretograms are given. The numbers indicate the days of the experiment.

Earlier electrophoretic examinations (9) suggested that there was sometimes an accumulation of lipid near the starting point. This was not confirmed using the electrophoretic techniques mentioned above. Identical findings were a broad beta band followed by a marked trail and a hardly detectable alpha band. There were no distinct changes during the different diets. However there were also some suspicious alterations. In paper electrophoresis there was a more easily detectable alpha band, and moreover just before the broad beta band, a further weak band (Fig. 2). By agaros gel electrophoresis the application band was weakest t the end of the high-fat week, corresponding in time with the smallest top fraction on the PVP density gradient (Fig. 3)

Only very small amounts of alpha-lipoprotein could be estimated quantitatively (precipitation, ultracentrifugation) during all dietary experiments. There were no distinct increases or decreases of alpha-lipoprotein corresponding to the changes of the plasma triglycerides. In study *A* the alpha-lipoproteins in the ultracentrifuge did not exceed 6 mg/100 ml, either during the high carbohydrate or during the high fat diets expressed in amount of cholesterol. In study *B* the alpha-lipoproteins after precipitation of the beta lipoproteins were 7–17 mg/100 ml during the high-fat period, and 14–20 mg/100 ml on the carbohydrate-rich diet expressed in amount of cholesterol.

Total plasma cholesterol decreased during the high fat diet and increased again when the patient received the high carbohydrate diet. The minimum and maximum plasma cholesterol concentrations were about 300 and 360 mg/100 ml in experiment *A*. The corresponding values in experiment *B* were about 305 and 380 mg/100 ml. No distinct change of the esterified part of the plasma cholesterol was seen. It varied from 27 to 32% independent of the diet.

The patient's weight did not change significantly in any of the experiments.

DISCUSSION

The dietary experiments clearly demonstrated that the lipemia in LCAT deficiency is carbohydrate-induced. The most remarkable result of a very high carbohydrate diet was the appearance of a large top fraction on the PVP density gradient.

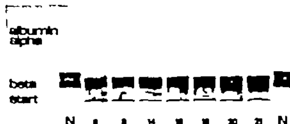


Fig. 3. Agaros gel electrophoretograms of the plasma lipoproteins during the dietary study *B*. For comparison two normal electrophoretograms are given. The numbers indicate the days of the experiment.

This fraction was diminished during the high fat diet. The top fraction is known to be the place for the exogenous triglycerides, while the endogenous seem to be located in the middle and bottom fractions (2, 8). This is the normal distribution, but the present work shows that there are exceptions from the rule. In LCAT deficiency triglyceride-rich lipoproteins are synthetized during a high carbohydrate diet with particular physical properties. On the PVP density gradient they behave as chylomicrons. However they differ from chylomicrons because they move on the agaros gel and paper electrophoreses. Obviously these lipoproteins are larger and lighter than normal endogenous VLD lipoproteins, possibly caused by the changed lipids.

In spite of low heparin-induced LLA there was no fat-induced lipemia (type I according to *Fredrickson et al.'s scheme (6)). The enzyme* activity is about the same in this disorder as in LCAT deficiency. The primary cause of the fat induced lipemia has been supposed to be the *reduced heparin-induced LLA (6). However from* the findings in LCAT deficiency it seems that this low enzyme activity may occur without induction of lipemia by fat.

Alpha-lipoprotein could hardly be detected in plasma independent of the degree of lipemia. Normally alpha-lipoprotein increases during diminishing triglyceridemia (14). Such changes could not distinctly be seen here. The constant low alpha-lipoprotein concentration is sometimes of the same degree as in familial high density lipoprotein deficiency (Tangier disease). However the findings in LCAT deficiency need not *mean that there is real alpha-lipoprotein de*ficiency. Like the triglyceride-rich lipoproteins

the alpha-lipoproteins may also have altered electrophoretic mobility on account of changed lipid content. The earlier reported decreased ratio cholesterol/phospholipid in VLD and particularly LD lipoproteins (9) possibly indicates that high density lipoprotein may be hidden in these lipoproteins. Immunological studies of the protein component of the lipoproteins are needed to clarify these problems. Such studies are in progress.

The occurrence of unusual triglyceride rich lipoproteins and possibly alpha-lipoprotein in LCAT deficiency need not necessarily mean that these lipoproteins are pathological. Another possibility is that they represent normal intermediate stages whose further changes are blocked by the lack of LCAT activity.

ACKNOWLEDGEMENT

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POLYMORPHONUCLEAR LEUCOCYTES IN THE SPECIFIC ANTIGEN-INDUCED INHIBITION OF THE IN VITRO MIGRATION OF HUMAN PERIPHERAL LEUCOCYTES

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Abstract. Antigen-induced inhibition of cell migration, a good in vitro parameter for cellular hypersensitivity, occurs only when the tested cell population contains both lymphocytes and cells with phagocytic properties. Leucocyte migration studies are presented of non-separated leucocytes, isolated polymorphonuclear leucocytes, isolated mononuclear leucocytes, a mixture of preseparated polymorphonuclear and mononuclear leucocytes, and a mixture of preseparated polymorphonuclear leucocytes and lymphocytes from brucella-positive and brucella-negative persons. Isolated polymorphonuclear leucocytes and isolated mononuclear leucocytes from brucella-positive persons did not show antigen-induced inhibition of migration. A mixture of preseparated polymorphonuclear and mononuclear leucocytes and a mixture of preseparated polymorphonuclear leucocytes and lymphocytes showed antigen-induced inhibition of migration to the same degree as non-separated leucocytes. The studies indicate that inhibition of leucocyte migration is dependent on the presence of both lymphocytes and polymorphonuclear leucocytes, whereas the monocyte scarcely plays any decisive part in the leucocyte migration test.

In a number of in vitro tests for assessing humoral and cellular hypersensitivity an antigen-induced cellular response occurs only when the tested cell population contains both lymphocytes and cells with phagocytic properties.

Investigation into the antigen-induced blastogenic response of lymphocytes has shown that "purified" lymphocytes not containing cells with phagocytic properties do not transform to blast cells (5, 6, 8). An admixture of leucocytes with phagocytic properties (8) or macrophages (6) restores the capacity of lymphocytes to transform in the presence of antigen toward which the individual has established hypersensitivity.

Similarly it has been shown that a secondary humoral immune response in vitro judged by the formation of antibodies and the development of plaque-forming cells in leucocyte cultures is substantially impaired when the number of cells with phagocytic properties falls below 4% (7).

An investigation of the antigen-induced inhibition of cell migration, a good in vitro method for estimating cellular hypersensitivity, has shown both in the case of animals (1, 4) and humans (11) that an inhibition reaction occurs only when lymphocytes as well as cells with phagocytic properties are present, whereas pure lymphocytes (4, 11) and pure macrophages (1) show no antigen-induced inhibition reaction.

The object of the present investigation has been to elucidate these reactions further through tests on human peripheral leucocytes.

MATERIAL AND METHODS

The material comprised 20 brucella-positive and 25 brucella-negative persons.

Brucella-positive persons are defined as those with a leucocyte migration index of less than 0.78 with an antigen concentration of 25 million brucella bacteria per ml.

Brucella-negative persons are defined as those with a migration index of ≥ 0.78 and a cutaneous reaction of less than 5 mm 48 hours after intracutaneous injection of 0.1 ml suspension of dead brucella bacteria (20 million bacteria per ml).

In this laboratory Søborg (10) has shown that a migration index of 0.78 distinguishes brucella-positive from brucella-negative with an antigen concentration of 40 million brucella bacteria per ml, but as the test has since been more sensitive the antigen concentration has been reduced to 25 million bacteria per ml.

The brucella-positive group consists partly of spontaneously positive persons and partly of persons vaccinated with 600 million dead brucella bacteria (Brucella abortus Bang).

Table I. The composition of the various cell populations used in the leucocyte migration test. The content of polymorphonuclear and mononuclear leucocytes expressed as a percentage (mean ± standard deviation and ranges)

Cell population	PMN	MN	MN with phagocytic capacity
PMN isolated by centrifugation ad modum Böyum	99.1±0.8 (96.0–99.8)	0.9±0.8 (0.2–4.0)	—
MN isolated by centrifugation ad modum Böyum	1.7±1.9 (0–5.0)	98.3±1.9 (95.0–100)	9.0±2.0 (8.0–12.0)
Ly isolated by the use of cotton-wool columns	0.7±0.8 (0–2.0)	99.3±0.8 (98.0–100)	0.16±0.10 (0–0.25)
Non-separated leucocytes	69±11 (38–88)	31±11 (12–62)	—
PMN + MN mixture of preseparated cells	64±9 (35–78)	36±9 (22–65)	—
PMN + Ly mixture of preseparated cells	68±8 (54–74)	32±8 (26–46)	—

Production of suspensions of pure polymorphonuclear leucocytes, mononuclear leucocytes (mixture of lymphocytes and monocytes) and lymphocytes

Polymorphonuclear leucocytes (PMN) and mononuclear leucocytes (MN) are separated by method described by Boyum (7). Leucocyte-containing plasma from heparinized blood spontaneously sedimentated for 1 hour at 37°C is diluted with an equal volume of Hank solution. Six to eight ml of this diluted leucocyte suspension is applied carefully to 3 ml Isopaque-Ficoll solution in 10 ml polysterol tubes. Isopaque Ficoll solution contains 10% Isopaque® (Nyegaard & Co., Oslo) and 6.4% Ficoll (Pharmacia, Uppsala) in water the specific gravity of the solution being 1.077. The tubes are centrifugated for 40 min at 400 g at room temperature. After centrifugation the MN form a white deposit in interphase between the plasma and Isopaque-Ficoll solution. This cell layer is removed with siliconized pipette. PMN which lie at the bottom of the centrifugating tube, are removed after resuspension.

Lymphocytes (Ly) are isolated with the aid of cotton-wool columns. Leucocyte-containing plasma is incubated in the column for 30 min at 37°C. In this way the PMN and monocytes are bound to the cotton-wool, whereas the lymphocytes can be drained off. The cotton-wool columns are washed through with a mixture of equal parts plasma and Hank's solution.

The purity of the isolated PMN, MN and Ly is assessed by differential count of 600 cells on May-Grünwald-Giemsa coloured smears.

Investigation of inhibition of leucocyte migration

In the present investigation the migration has been examined of non-separated leucocytes, PMN, MN, mixture of preseparated PMN and MN and mixture of preseparated PMN and Ly. The migration test was carried out as described in detail by Søborg and Bendixen (9). The leucocytes were washed thoroughly and drawn into capillary tubes which were placed in 1 ml culture chambers. Inside half of the chambers 25 million dead brucella bacteria were placed. The migration area was measured after 4 hours. The mean area in antigen-containing cultures divided by the mean area in antigen-free cultures was called the migration index (MI). The MI is a measure of the antigen-induced inhibition: the stronger the inhibition the lower the MI.

Examination of phagocytosis

Three-hundred μl suspension of leucocytes in autologous plasma with approx. 5,000 cells per μl is mixed with 30 μl 0.5% suspension of latex particles (Dow Latex, diameter 1.947 μm) in Hank' solution. The mixture is incubated for 30 min at 37°C, after which the cells are smeared and coloured with May-Grünwald-Giemsa. The relative number of cells with intracytoplasmic latex particles is counted.

RESULTS

Table I shows the composition of the various cell populations investigated in the leucocyte migration test.

Table II shows the results of an investigation of the migration inhibition of non-separated leucocytes, isolated PMN as well as a mixture of preseparated PMN and MN from 20 brucella positive and 20 brucella-negative persons.

The mean MI for non-separated leucocytes shows a significant difference between the brucella-positive and the brucella-negative group ($p < 0.001$), whereas the mean MI for PMN shows no difference between the two groups ($p > 0.1$). In none of the groups is a correlation found between the MI for non-separated leucocytes and the MI for isolated PMN from the same person ($r = 0.32$, $p > 0.1$ for brucella positive, $r = 0.37$, $p > 0.1$ for brucella-negative).

In neither the brucella-positive nor the brucella-negative group is there any statistically significant difference between the mean MI for non-separated leucocytes and the MI for a mixture of preseparated PMN and MN from the same persons ($p > 0.1$).

In no case was the migration of a mixture of preseparated PMN and MN from brucella negative inhibited by antigen. In the case of the brucella-positive Fig. 1 indicates that there is good correlation between the MI for non-separated leucocytes and the MI for a corresponding

Table II. *Migration indices of non-separated leucocytes, polymorphonuclear leucocytes and mixtures of pre separated polymorphonuclear and mononuclear leucocytes from 20 brucella-positive and 20 brucella-negative persons*

Brucella-positive persons			Brucella-negative persons		
Non-sep. leucocytes	PMN	Mixture of presep. PMN and MN	Non-sep. leucocytes	PMN	Mixture of presep. PMN and MN
0.56	0.81	0.60	0.82	0.82	—
0.57	0.85	0.56	0.83	0.80	0.94
0.59	0.85	0.66	0.83	0.83	0.83
0.60	0.80	0.68	0.84	0.86	0.83
0.65	0.83	0.64	0.84	0.80	0.97
0.65	—	0.69	0.85	0.89	0.89
0.67	0.81	0.64	0.86	0.82	0.81
0.67	0.83	—	0.87	—	0.90
0.67	0.86	0.62	0.87	0.92	0.96
0.68	0.82	0.69	0.87	0.93	0.90
0.68	0.84	0.67	0.88	0.87	0.89
0.69	0.79	—	0.89	0.90	0.89
0.70	0.88	—	0.89	0.91	0.92
0.72	0.86	0.67	0.94	0.98	0.95
0.72	0.96	0.73	0.97	0.80	—
0.72	0.97	0.76	0.98	0.81	—
0.74	0.87	0.70	1.02	0.95	—
0.74	0.88	0.75	1.03	0.92	0.94
0.74	0.79	—	1.04	0.93	0.85
0.77	0.83	0.71	1.09	0.87	0.93
Mean					
0.68	0.85	0.67	0.91	0.87	0.90
S D					
0.06	0.05	0.05	0.08	0.06	0.05

mixture of preseparated PMN and MN from the same person ($r = 0.75$ $p < 0.001$).

Table III shows the results of an investigation of the inhibition of migration of non-separated leucocytes and isolated MN from eight brucella-positive and six brucella-negative persons.

The mean MI for non-separated leucocytes shows a significant difference between the brucella-positive and brucella-negative group ($p < 0.001$), whereas the mean MI for MN shows no difference between the two groups ($p > 0.1$).

There is no correlation between the MI for non-separated leucocytes and the MI for isolated MN from the same person either in the brucella-positive ($= 0.35$ $p > 0.1$) or the brucella-negative group ($= 0.25$ $p > 0.1$).

Table IV shows the results of an investigation of the inhibition of migration of non-separated leucocytes, PMN MN and mixtures of preseparated PMN and MN or Ly from five brucella-positive persons.

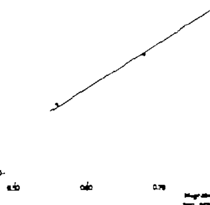


Fig. 1. Correlation of the migration index of non-separated leucocytes and that of mixture of preseparated polymorphonuclear and mononuclear leucocytes from 16 brucella-positive persons.

Table III *Migration indices of non-separated leucocytes and mononuclear leucocytes from eight brucella-positive and six brucella-negative persons*

Brucella-positive persons		Brucella-negative persons	
Non-sep. leucocytes	MN	Non-sep. leucocytes	MN
0.67	0.96	0.87	0.98
0.68	0.93	0.88	0.86
0.68	0.86	0.92	0.99
0.69	0.88	0.93	0.88
0.72	1.04	0.95	0.87
0.72	0.88	0.99	0.91
0.74	0.95		
0.74	0.96		
Mean			
0.71	0.93	0.92	0.92
S.D			
0.03	0.06	0.04	0.06

The mean MI for non-separated leucocytes, a mixture of PMN + MN and a mixture of PMN + Ly does not show any statistically significant difference between these three cell populations ($p > 0.1$), whereas the three cell populations all have a mean MI significantly different from the mean MI for both isolated PMN and isolated MN ($p < 0.005$)

Fig 2 shows diagrammatically the combined results of migration investigations with different leucocyte populations from brucella-positive persons. It will be seen that isolated PMN and isolated MN do not show antigen-induced inhibition of migration, whereas inhibition is clearly present for non-separated leucocytes, a mixture of preseparated PMN and MN and a mixture of preseparated PMN and Ly

Fig. 3 shows corresponding results of migration investigations of different leucocyte populations from brucella-negative persons.

Table IV *Migration indices of non-separated leucocytes polymorphonuclear leucocytes mononuclear leucocytes and mixtures of preseparated polymorphonuclear leucocytes and mononuclear leucocytes or lymphocytes from five brucella-positive persons*

Non-sep. leucocytes	PMN	MN	Mixture of presep. PMN and MN	Mixture of presep. PMN and Ly
0.67	0.86	0.96	0.62	0.72
0.68	0.82	0.93	0.69	0.67
0.72	0.97	0.88	0.76	0.73
0.74	0.87	0.95	0.70	0.75
0.74	0.88	0.96	0.75	0.68
Mean				
0.71	0.88	0.94	0.70	0.71
S.D				
0.03	0.06	0.03	0.06	0.03

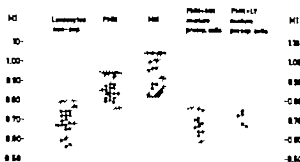


Fig 2. Leucocyte migration test on various leucocyte populations from brucella-positive persons. The shaded area represents mean ±2 S.D Figures presented in Tables II, III and IV

DISCUSSION

In experiments with tuberculin-positive guinea pigs, David (4) indicates that a population of lymph node cells, consisting mainly of Ly does not show antigen-induced inhibition of migration, while a population of peritoneal exudate cells, containing approx. 15% Ly and the rest mainly macrophages, does show antigen-induced inhibition.

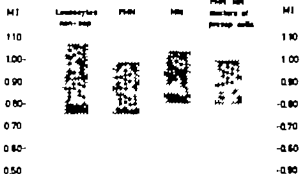


Fig 3 Leucocyte migration test on various leucocyte populations from brucella-negative persons. The shaded area represents mean ±2 S.D Figures presented in Tables II and III.

A mixture of 15–35% lymph node cells from tuberculin-positive guinea-pigs and 85–65% peritoneal exudate cells from non-sensitive animals shows distinct inhibition of migration. Absence of inhibition for pure lymph node cells is thus not attributable to the fact that these cells do not contain sensitive Ly

Similarly Søborg (11) has shown that the migration of pure human Ly from brucella-positive persons is not inhibited by antigen, while a mixture of these sensitive Ly and non-separated leucocytes from a brucella-negative person does show antigen-induced inhibition.

Bloom and Bennett (1) have examined peritoneal exudate cells from tuberculin-positive guinea-pigs and separated macrophages and Ly The macrophages showed no antigen-induced inhibition of migration, while a mixture of macrophages and Ly showed distinct inhibition. The purity of the macrophages in these experiments was between 99.4 and 99.8%

These experiments indicate that both Ly and cells with phagocytic properties are necessary in order to achieve an antigen-induced inhibition f migration.

The results of the present migration experiments with human leucocytes confirm Søborg's investigations, which demonstrated that antigen-induced inhibition of migration requires the presence of both Ly and cells with phagocytic properties. It is further shown that isolated PMN and isolated MN (a mixture of about 90% Ly and 10% monocytes) from brucella-positive persons do not show antigen-induced inhibition.

The absence of antigen-induced inhibition in the case both of PMN and MN from brucella positive persons could be due to damage of the cells during the separation process. Böyum however (2), who provided the technique used for this separation, found that the separated cells appeared normal were of normal viability judged by vital colouring, and that the phagocytes had normal capacity for phagocytosis.

That mixture of preseparated PMN and MN from brucella-positive persons shows antigen-induced inhibition of migration to the same extent as corresponding non-separated leucocytes indicates clearly that neither PMN nor MN is damaged in connexion with this cell function.

The antigen-induced inhibition of a mixture of preseparated PMN and MN from brucella-positives is specific, since migration of a mixture of preseparated PMN and MN from brucella-negatives is not inhibited by antigen.

The absence of antigen-induced inhibition in pure PMN corresponds to what Bloom and Bennett (1) found for pure peritoneal macrophages from sensitive guinea-pigs.

A number of investigations of antigen-induced lymphocyte transformation (3 6) indicate that monocytes play a part in the transformation, and since peritoneal macrophages, which are the phagocytic cells used in most animal experiments, are presumably derived from the blood monocytes, it is conceivable that monocytes also play a part in the leucocyte migration test.

In the present investigation pure MN from brucella-positive persons did not show antigen-induced inhibition of migration. Pure MN comprises about 90% Ly and 10% monocytes, and this surplus of Ly may explain the bsence of antigen-induced inhibition. Preliminary investigations in this laboratory have shown that a mixture of 85–90% sensitive Ly and 10–15% PMN does not show antigen-induced inhibition either This observation corresponds to what David (4) showed experimentally in animals, that a mixture of 20% sensitive peritoneal exudate cells and 80% spleen Ly from non-sensitive animals does not show antigen-induced inhibition of migration, whereas a mixture of 20% sensitive peritoneal exudate cells and 80% non-sensitive peritoneal exudate cells shows distinct antigen-induced inhibition. Thus, in these animal experiments, a cell population containing sensitive Ly shows antigen-induced inhibition only when the cell population also contains considerabl number of macrophages.

Non-separated leucocytes and a corresponding mixture of preseparated PMN and MN contain 3–4% monocytes. In order to ascertain whether this monocyte content together with PMN plays any part in the leucocyte migration test, the MI for non-separated leucocytes and corresponding mixture of preseparated PMN and MN was compared with the MI for corresponding mixture of preseparated PMN and Ly from the same brucella-positive persons. The MI for these three leucocyte populations showed equally marked antigen-induced inhibition of migration.

The tested mixture of preseparated PMN and Ly contained very few monocytes Isolated PMN

In these experiments contained only between 0.2 and 0.5% MN and isolated Ly only 0 16% MN with phagocytic properties. The combined monocyte content was thus well below 1%

The present investigation shows that with the cell combination existing in non-separated leucocytes and corresponding mixtures of preseparated PMN and MN PMN together with Ly is decisive for antigen-induced inhibition of migration, whereas the monocyte scarcely has any independent importance. This does not exclude the possibility however that monocytes may take the place of PMN if monocytes and Ly are mixed in the same proportion as that for PMN and Ly in non-separated leucocytes.

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TOTAL EXCHANGEABLE SODIUM IN CHRONIC NEPHROPATHY WITH AND WITHOUT HYPERTENSION

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Abstract. The total exchangeable sodium has been determined by the isotope dilution technique with ^{24}Na in 1) 17 normal subjects; 2) 13 patients with essential hypertension and normal renal function; 3) 21 patients with renal failure without hypertension; and 4) 22 patients with renal failure with hypertension. In the last two groups there were respectively nine and eight patients who received intermittent dialysis treatment. The total exchangeable sodium was normal in all patients of groups 1 and 2, it was elevated in 14.3% of the patients in group 3 and in 63.5% of the patients in group 4. The mean value was significantly higher in group 4 than in group 3. Total exchangeable sodium was elevated in all patients with renal failure, hypertension and peripheral oedema. An elevation was found more frequently in patients with renal failure *and* peripheral oedema than in patients with renal failure *without* manifest oedema. Likewise, in patients with renal failure *and* hypertension an elevated value of exchangeable sodium was found more frequently than in patients with renal failure *without* hypertension. On the other hand, no correlation was found between the presence of oedema and the presence of hypertension in the above patients with renal failure. During dialysis treatment an existing increased exchangeable sodium became normal, except in one patient.

Total exchangeable sodium (TES) represents the physiologically and osmotically active sodium in the organism. It constitutes about 75% of the total sodium, as the sodium content of the bones is exchangeable to only a very slight degree (Moore et al. (20)). Using the isotope dilution method (^{24}Na) there have been relatively few studies on the magnitude of the exchangeable sodium in patients with renal failure, and in particular on the influence of dialysis on this magnitude in such patients. Blumberg et al. (3) have examined a total of 14 chronically uraemic patients, 6 of whom underwent intermittent dialysis treatment. A certain relationship was demonstrated between the magnitude of the arterial blood pressure and TES, although it was not possible to exclude other causes of the hypertension. Funck Brentano et al. (11) and Comty et al. (6) reached similar results, measuring TES in respectively 17 and 9 chronically uraemic patients.

MATERIAL

TES was determined in a patient material of 1) 17 normal subjects; 2) 13 patients with essential, benign hypertension with normal serum creatinine concentration and without demonstrable renal disease; 3) 21 patients with renal failure without hypertension, nine of whom (group 3 *b*) received intermittent dialysis treatment; and 4) 22 patients with renal failure with hypertension, eight of whom (group 4 *b*) received dialysis treatment. Table I shows the sex, age distribution, weight distribution, serum creatinine concentration and diagnosis. Arterial hypertension is taken to mean a condition in which the mean arterial blood pressure (diastolic pressure +1/3 of the amplitude) exceeds the limits established by Master et al. (16). The age distribution was uniform in the different groups, apart from the fact that the hypertensive patients who received dialysis treatment (group 4 *b*) were slightly younger. The mean weight was the same in all groups, apart from a lower value in group 4 *b* than in group 2 ($0.02 > p > 0.01$). The mean serum creatinine concentration determined at the time of investigation was the same in three of the groups of uraemic patients. The non-dialyzed, non-hypertensive patients had somewhat lower values.

With respect to the diagnoses, relatively most cases of glomerulonephritis occurred in the group of hypertensive, nondialyzed patients with uraemia (group 4 *a*).

The majority of the non-dialyzed patients with uraemia had not been subjected to any dietary restriction of sodium, while the dialyzed patients received a diet containing 40–50 g protein and about 10 mEq sodium per 24 h.

Table I *Clinical data*

Group	No. of pats.	Sex ♂	Sex ♀	M (y	Mean weight (kg)	Mean serum creatinine concentration (mg/100 ml)	Diagnosis		
1	17	4	13	4	61 7 (45–90)	0.88 (0.7 1.3)	Normal subjects		
2	13	6	7		71 6 (48–103)	1.07 (0.8–1.3)	Hypertensio arterialis essentiais benigna		
							Pyelonephritis chron.	Glomerulonephritis chron.	Varia
3	12	3			61 1 (36–85)	6 21 (1 6–12.6)	6	1	5
3b	9	1			60.6 (41 101)	8.81 (2.2 15 7)	6	2	1
4	14	8			61 9 (44–91)	8.88 (2.2 18.2)	5	6	3
4b	8	3			52.8 (26–68)	8.57 (4 6–11 8)	5	2	1

Figures in bracket e

A determination f TES [illegible] ients, using the method described [illegible] (18), with 24-hour equilibra[illegible] 50–100 μC Na CO labelled [illegible] activity of approximately 200 mC [illegible] usly and the concentration [illegible] N in the serum as determined [illegible] pressed as percentage f the [illegible] per l serum. At the same time [illegible] d sample containing 1 100 of the dose [illegible] A total of at least 2,000 counts as recorded [illegible] made in well scintillation crystal dete[illegible] able sodium as determined by flame pho[illegible] ient of variation 1...) At the same time the [illegible] radioactiv[illegible] sodium excreted in the [illegible] first 4 hours after the injection of the radioactive [illegible] as measured and expressed as a percentage of the dose administered. The residual radioactivity in the injection syringe was always determined, and this value was subtracted from the radioactivity in the syringe prior to injection.

TES was then calculated from the equation

TES (mEq) =

(dose injected—dose excreted (24 h)) in [illegible] of total dose / [illegible]Na in serum in % of dose/l serum/[illegible]Na in serum in mEq/l serum

According to several author (21) an equilibration period of 24 hours is adequate to permit determination of practically all exchangeable sodium, and it is possible that reliable results can be obtained even after shorter period of equilibration (24). In the present investigation the results are expressed in mEq per kg body weight. The reports of normal values vary somewhat in the different patient materials, being somewhat higher for men than for women, since the latter contain relatively more fat (e.g. Forbes and Perley (10) ♂ 32.3–54 1 mEq/kg, and ♀ 35 7–41 6 mEq/kg; Miller and Wilson (18) ♂ 39.3–47.9 mEq/kg, and ♀ 39 4–44 8 mEq/kg, and Moore et al. (19) ♂: 36.1–48.3 mEq/kg, and ♀ 34.4–45.9 mEq/kg). No age dependence has been found. On the other hand, the values expressed per kg are reported to be somewhat lower in obese subjects (12). We have found coefficient f variation of 5%. The serum creatinine concentration was determined by the method of Bonsnes and Taussky (4) (coefficient of variation 5% normal value ♂ < 1.3, and ♀ < 1.2 mEq/100 ml). Finally the endogenous 24-hour creatinine clearance was determined in 41 out of the 43 patients with uraemia.

In the 43 patients of groups 3 and 4 total of [illegible] determinations were made of the total exchangeable sodium. In those patients in whom several determinations were made, only the first determination has been included in the analysis.

In groups 3 and 4 a total of 17 patients underwent intermittent dialysis treatment, in part peritoneal dialysis as described by Larsen et al. (15), in part haemodialysis by the method of Scribner Kill (see Bandler et al. (1)).

The selection of the non-dialyzed patients in the present investigation was determined by their presence in the department on that day of the week when the radioactive sodium was delivered.

RESULTS

Table II and Figs. 1 and 2 show the mean blood pressure, the serum sodium concentration and the TES/kg. There was no significant difference between the mean blood pressures in groups 1 and 3 i.e. the groups without hypertension, just as

there was no difference in the mean blood pressure between the groups with hypertension (groups 2 and 4). On the other hand, the mutual differences between groups 1 and 2 and between groups 3 and 4 were highly significant ($p < 0.001$, $p < 0.001$).

The findings with respect to serum sodium concentration were as follows: in groups 1 and 2 the values for serum sodium concentration were within normal limits and the mean concentration was the same. In groups $3\,a+b$ and $4\,a+b$ the mean values for the serum concentration were somewhat lower than for groups 1 and 2, and the difference was significant in the case of the dialyzed groups (3 *b* and 4 *b*) ($p < 0.01$).

In groups 3 *a* and 3 *b* and 4 *a* and 4 *b* there were respectively 2, 3, 5 and 3 patients with values below the lowest normal limit (134 mEq/l).

The following values were found for the magnitude of TES/kg:

In group 1 normal values for men 37.3–53.7 mEq/kg, mean value 45.5 mEq/kg, for women 36.9–48.1 mEq/kg, mean value 42.5 mEq/kg, which are identical with those found by Farber and Soberman in a normal material of 27 persons (8). In group 2, the mean value for TES/kg was identical with the mean value in group 1 just as values above the normal range were not found in group 2. In three cases (23 %) of this group slightly reduced values were found.

Table II. *Average mean blood pressure, serum sodium concentration and total exchangeable sodium*

Group	Mean blood pressure (mm Hg) (±S.D.)	Mean serum sodium concentration (mEq/l) (normal 134–152) (±S.D.)	Mean TES (mEq/kg) (±S.D.)
1	94.6±15.5 (68–123)	141.2±3.2 (134–147)	43.2±3.9 (37.9–51.0)
2	148.8±10.0 (135–164)	141.2±2.7 (136–145)	42.6±6.3 (34.0–53.0)
3	102.6±17.5 (73–130)	138.3±5.1 (129–145)	44.8±8.2 (31.6–63.0)
3 *b*	104.3±16.4 (73–133)	134.4±5.2 (122–139)	46.3±4.0 (34.6–51.5)
4	153.9±16.0 (127–193)	136.2±8.7 (119–147)	50.4±9.0 (34.6–64.0)
4 *b*	135±26.1 (117–180)	135.5±3.4 (130–139)	60.2±15.9 (35.5–84.5)

Figures in brackets: extreme values.

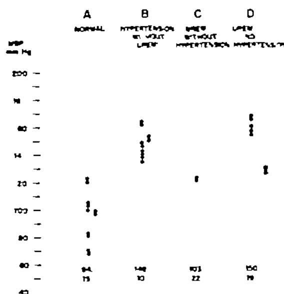


Fig. 1. Mean blood pressure in the various patient groups: O patients treated with dialysis.

In group 3 (normotensive patients with renal failure) the mean value of the exchangeable sodium was not found to differ significantly from the mean values in groups 1 and 2. It should be mentioned, however, that in group 3 there were three out of 21 patients (14.3 %) with elevated exchangeable sodium, two out of 12 in group 3 *a*, and one out of nine in group 3 *b* (16.7 % and

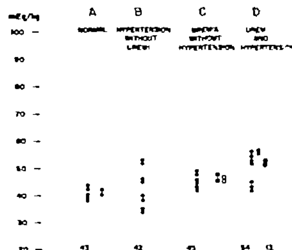


Fig. 2. Total exchangeable sodium in the different patient groups: O patients treated with dialysis.

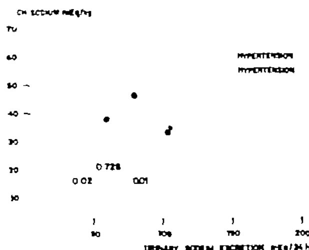


Fig 3 Relation between total exchangeable sodium and sodium excretion in the urine.

11 1% respectively) Two patients had slightly reduced values (9.5%).

In the case of group 4 (hypertensive patients with renal failure) the mean value for TES/kg was found to be significantly higher than for group 3 ($t = 2.37$ $0.05 > p > 0.0?$). Comparing groups 3 *b* and 4 *b* the mean value in groups 4 *b* is significantly higher than in group 3 *b* ($t = 2.29$ $0.05 > p > 0.0$), whereas there is no significant difference between groups 3 *a* and 4 *a* (the non-dialyzed patients) as $t = 1.68$ $p > 0.1$ However there was a definitely higher mean value for TES/kg in group 4 *a* (non-dialyzed hypertensive patients) than in groups 2 and 1 (hypertensive patients without renal failure and the normal group), as the *t* and *p* values are 2.58, $0.0? > p > 0.01$ and ..96, $0.01 > p > 0.001$ respectively

In group 4 as a whole two patients had slightly reduced values (9 1%) while 14 out of 22 patients had elevated values for the exchangeable sodium (63.5%). This is significantly different from the value of 14.3% in group 3 ($\chi^2 = 10.19$ $0.01 > p > 0.001$) Of the non-dialyzed hypertensive patients with renal failure (group 4 *a*), eight out of 14 had an elevated value of exchangeable sodium (57.2%). This also differs significantly from the value for the corresponding group of normotensive patients (group 3 *a*) as $\chi^2 = 4.4..$, $0.05 > p > 0.02$. Six out of eight patients in group 4 *b* had increased exchangeable sodium (75%)

Factors Possibly Influencing the Magnitude of the Exchangeable Sodium in Patients with Impaired Renal Function

A. Excretion of sodium in the urine

Fig. 3 shows the relationship between the magnitude of the exchangeable sodium and the 24-hour excretion of sodium in the urine of 11 patients with renal failure who had not received treatment with sodium or diuretics. There is a significant negative correlation ($0.02 > p > 0.01$) and the rela

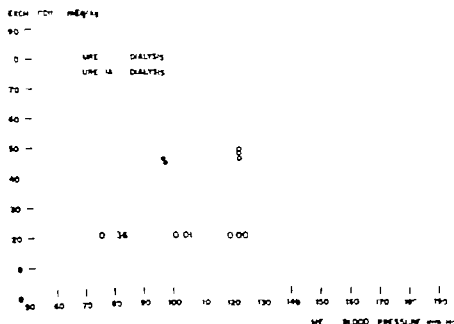


Fig 4 Relation between total exchangeable sodium and mean blood pressure

Table III. *Influence of loss of sodium, supplement of sodium and treatment with diuretics on the exchangeable sodium*

		No. of patients			
Group	TES	Total	Heavy loss of sodium in urine	Supplement of sodium prior to investigation	Treatment with diuretics prior to investigation
3	Increase	3	0	1	0
	No increase	18	1	2	2
4	Increase	14	0	2	2
	No increase	8	2	0	1

tionship is defined by the equation for the regression line $y = -0.15x + 51.6$ $r = -0.728$.

B. *The level of the mean blod pressur*

For groups 3 and 4 as a whole a significant positive correlation was found between the magnitude of the exchangeable sodium and the mean blood pressure ($0.01 > p > 0.001$) The relationship is shown in Fig. 4 and the regression line is defined by the equation $y = 0.16x + 29.58$, $= +0.436$.

Examining solely the group of hypertensive patients with renal failure, no definite relationship is seen here between exchangeable sodium and the mean blood pressure ($p > 0.1$ $= +0.133$)

C. *Endogenous 24-hour creatinine clearance*

The greatest measured value of the endogenous 24-hour creatinine clearance in groups 3 and 4 was 34 ml per min, and 32 out of the 41 patients examined in these groups had clearance values below 10 ml per min. No relationship was demonstrated between the magnitude of the exchangeable sodium and the endogenous 24-hour creatinine clearance in the patients with chronic renal failure ($p > 0.1$ $= -0.182$).

D *Sodium intake or treatment with diuretics*

Table III shows that in group 3 two out of 18 patients with normal values of exchangeable sodium had been treated with diuretics of the chlorthiazide group, and two had received large supplements of sodium (150–200 mEq/24 h). Of the three patients with increased exchangeabl sodium in the same group one had received a supplement of sodium prior to the investigation.

In group 4 one out of eight patients with normal exchangeable sodium had been treated with furosemide (Lasix®), while two had a strikingly high urinary excretion of sodium without any definite explanation. Of the 14 patients with increased exchangeable sodium in the same group two had received large supplements of sodium, while a further two patients had been treated with diuretics.

Thus sodium intake or treatment with diuretics can hardly have had any decisive influence on the distribution of the patients in the groups with normal or increased exchangeable sodium. In the latter group, however no patients were found with abnormally high sodium loss in the urine.

E. *Dialysis treatment*

During the course of the dialysis treatment repeated measurements of the exchangeable sodium were made in 11 out of the 17 patients treated with dialysis in groups 3 and 4 At the start of the investigation six of these 11 patients had either normal TES/kg (5 patients) or reduced TES/kg (1 patient) while five of the 11 patients had increased TES/kg.

Fig. 5 *a* and 5 *b* show the changes in TES/kg during the dialysis treatment in patients with normal or reduced TES/kg (Fig. 5 *a*) and in patients with elevated TES/kg (Fig. 5 *b*) The abscissa shows the number of dialyses, but not the duration of treatment.

Fig. 5 shows that during the dialysis treatment none of the six patients developed elevated values of exchangeable sodium. Patients with a subnormal value of TES/kg developed rising TES/kg, although the value never became completely normal. Fig. 5 *b* shows that apart from a single patient, who only received one dialysis

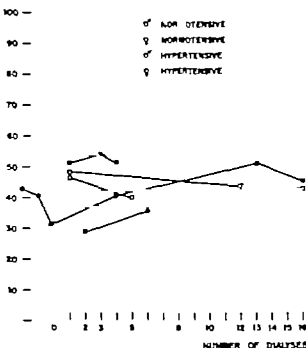


Fig 5 a. Relation between total exchangeable sodium and number of dialyses in uraemic patients in whom the total exchangeable sodium was normal or reduced before the dialyses. The figures give the number of dialyses before or between the measurements.

treatment, and who in addition had a sodium supplement, the initially elevated exchangeable sodium became normal during dialysis treatment in the other four patients. In one of the patients it rose once more to elevated values with insufficient dialysis treatment in the terminal phase of the disease (no.). There was seemingly no correlation between a possible fall in TES/kg and normalization of the mean blood pressure. However if the patients marked 1 to 3 are analyzed in greater detail with respect to the course of the mean blood pressure exchangeable sodium and serum creatinine during the course of the dialysis treatment, it is nevertheless seen that there was some agreement between the variations in mean blood pressure and the changes in TES kg, while the association with changes in the serum creatinine concentration was less pronounced (Figs. 6–8). It should be recalled here that in the dialyzed patients the serum creatinine concentration, precisely on account of the dialysis treatment, is not a satisfactory expression of the renal function.

F Fluid retention hypertension and total exchangeable sodium

As patients with cardiogenic oedema often have increased exchangeable sodium (2, 8), an attempt was made in the patients with renal failure (groups 3 and 4) to evaluate the relationship between possible oedema, hypertension and exchangeable sodium. Table IV shows the distribution of the normotensive patients and the patients with hypertensive renal failure according to the degree of the exchangeable sodium and the presence of oedema. Patients with decompensated heart disease are grouped separately since to make this diagnosis it is necessary that, in addition to the presence of peripheral oedema and/or oedema of organs, there should be clinical and cardiographic signs of hypertrophy and overloading of the left heart—possibly confirmed at autopsy In groups 3 and 4 a total of nine patients had signs of overloading of the left ventricle, with cardiac decompensation verified clinically and/or at autopsy Of the remaining 34 patients nine had oedema, while 25 had no manifest oedema at the time of investigation. It is seen that patients with

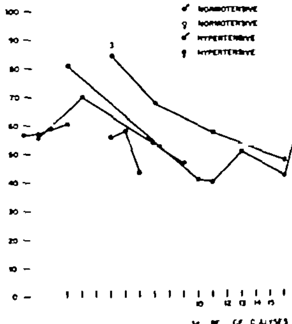


Fig 5 b Relation between total exchangeable sodium and number of dialyses in uraemic patients in whom the total exchangeable sodium was elevated before the dialyses. The figures give the number of dialyses before or between the measurements.

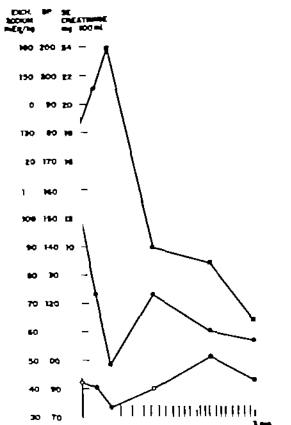


Fig. 6. Relation between total exchangeable sodium, mean blood pressure, serum creatinine concentration and time, in patient no. 1. Number of dialyses is indicated on the abscissa.

renal failure, arterial hypertension and oedema all had elevated TES/kg. Of the nine patients with oedema seven had elevated TES/kg. For comparison, only four out of the 25 patients without oedema had elevated TES/kg. The difference between the incidence of elevated TES/kg in these two groups is highly significant ($\chi^2 = 8.59$ $p < 0.001$).

Of the 34 patients with reduced renal function, a total of 15 patients had hypertension. Nine of these had elevated TES/kg. Only two of the 19 normotensive patients had elevated TES/kg, a difference which is likewise highly significant ($\chi^2 = 9.17$ $p < 0.001$).

Thus both hypertension and the presence of oedema are correlated with elevated TES/kg. Hypertension and oedema, on the other hand, are not definitely correlated mutually as a total of six patients out of 15 with hypertension had oedema, while three patients out of 19 without hypertension had oedema, a difference which is not significant ($\chi^2 = 2.45$ $p > 0.05$)

DISCUSSION

Our findings confirm that the total exchangeable sodium is normal in essential hypertension (13). The magnitude of the exchangeable sodium has thus no great significance for the genesis of the essential hypertension. The situation seems to be somewhat different in uraemia. We found the mean TES/kg significantly higher and elevated in 2/3 of the cases where hypertension was present,

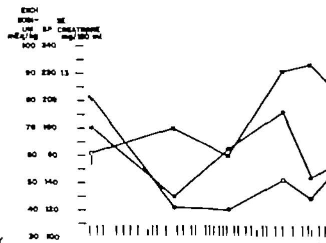


Fig. 7. Relation between total exchangeable sodium, mean blood pressure, serum creatinine concentration and time in patient no. 2. Number of dialyses is indicated on the abscissa.

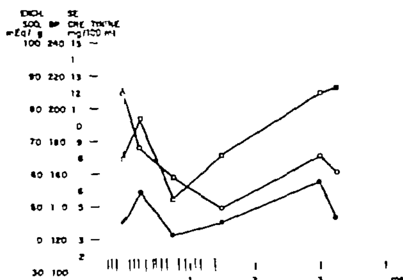


Fig. 8 Relation between total exchangeable sodium, mean blood pressure, serum creatinine concentration and time in patient no. 3 Number of dialyses is indicated on the abscissa.

while the exchangeable sodium was most often normal, provided the patients with uraemia did not have hypertension. A positive correlation was demonstrated between the level of the mean blood pressure and the magnitude of the exchangeable sodium. In agreement with our results, Carlberger and Collste (5) have published the results of a study of the relationship between the arterial blood pressure and TES/kg in 15 patients with chronic renal failure and an endogenous creatinine clearance below 3 ml/min, who were undergoing dialysis treatment. These authors also found a clear correlation between TES/kg and both systolic and diastolic blood pressure. This, of course, does not mean that the increased exchangeable sodium is the cause of the hypertension in these patients, even though it may be a contributory factor About 1 3 of the patients with hypertension had in fact normal values of exchangeable sodium. In this connection it is of interest that Kolff et al (14) have reported that patients with pronounced renal failure remained hypertensive, even though a sodium depletion developed, and that rats with clips on the renal arteries and hypertension (9) remained hypertensive when sodium was removed by peritoneal dialysis. There thus appeared to be several mechanisms in the development of hypertension in uraemia.

The reason for the increase in exchangeable sodium in certain patients with uraemia is unclear The excretion of sodium in the urine was examined in only 11 of the patients (Fig. 3). Only a weak negative correlation to the exchangeable sodium was found, so that it is hardly a *question of excretion alone. No correlation was* found to the degree of renal insufficiency

The possible significance of the so-called natriuretic hormone for the excretion of sodium is of interest (7 23) The excretion of sodium i

Table IV *Influence of oedema on exchangeable sodium*

		No of patients				
Group	TES	Total	Peripheral oedema without cardiac failure	No oedema or cardiac failure	Peripheral oedema and cardiac failure	Cardiac failure but no peripheral oedema
3	Increase	3	1	1	1	0
	No increase	18	2	15	1	0
4	Increase	14	6	3	5	0
	No increase	8	0	6	1	1
	Total no.	43	9	25	8	1

determined by the rate of glomerular filtration and by the reabsorption, which is controlled by the action of the mineralocorticoids on the renal tubules and of a so-called third factor on the tubule cells, a factor which is stimulated by an increase in extracellular volume, the effect of which is to increase the excretion of sodium. This factor does not appear to be effective in the presence of oedema, and the excretion of sodium is then reduced. It is of interest, therefore, that in the present patient material a clear correlation was demonstrated between the presence of oedema and an elevated TES/kg, even when one ignores patients with cardiac failure (Table IV).

Patients with renal failure, hypertension and oedema all had elevated TES/kg, but also when considered as whole a significant correlation was found between oedema and elevated TES/kg as well as between hypertension and elevated TES/kg.

No definite relationship was found between oedema and hypertension. In our patients we did not have the opportunity to determine the total water content at the same time, but in agreement with our clinical observations Carlberger and Collste (5) have been unable to demonstrate in their patients any correlation between the total water content of the body and the level of blood pressure.

In the present investigation no correlation was found between the serum sodium concentration and TES/kg. In particular uraemic patients with a low serum sodium concentration (a total of 13 patients) did not have a low TES/kg. On the contrary five out of the 13 had an elevated TES/kg. All these patients had severe uraemia and hypertension. A possible explanation is that the sodium pump fails in such cases, so that the distribution of sodium between the intracellular and the extracellular space is abnormal. Moore et al. (19) have obtained similar results.

A TES/kg value which was already elevated became normal in four out of five cases during the dialysis treatment. It should be mentioned here that in two of the patients the TES/kg value continued to be elevated at time when the mean blood pressure was normal. TES/kg and the mean blood pressure increased at later stage in the one patient when the effectiveness of the peritoneal dialysis decreased.

There has been some discussion as to the most suitable dietary treatment in patients with uraemia (17 22). The risk of the clinical picture becoming exacerbated due to the salt depletion syndrome, caused by a salt-poor regimen, must be weighed against the advantages which can apparently be achieved by such a regimen with respect to better blood pressure control and thereby retardation of the progression in the patient's renal failure.

The conclusion to be drawn from our studies must be that uraemic patients with hypertension and with manifest oedema should be subject to a salt-poor regimen, and if the TES/kg is elevated, a salt-poor regimen should be instituted even though no oedema is present. On the other hand, it does not seem possible to determine from the serum sodium concentration whether a salt-poor regimen is indicated or not.

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EVALUATION OF PANCREATIC SCANNING

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Abstract. Pancreatic scanning is evaluated. The scanning diagnosis was correct in 96% of patients whose scans were interpreted as normal. When scan was regarded as pathological, in only 47% could pancreatic disease be verified. The reason for obtaining wrong positive scans is not well understood. The present study would indicate that diseases of the liver and gastrointestinal tract will often cause diffuse decrease in the incorporation of ^{75}Se-selenomethionine into the pancreas. In patients with malignant disease or diabetes there were also many wrong scanning diagnoses. Pancreatic scanning is therefore of limited value in the diagnosis of diffuse pancreatic disease. It can readily be used for detecting pancreatic tumors of over 2 cm size. Its major value is, however, in excluding pancreatic disease with high degree of confidence when normal scan is obtained.

Radioisotope scanning of the pancreas is based on the demonstration of a high avidity of the pancreas for ^{75}Se-selenomethionine (2). The chemical properties of the internally labelled selenomethionine are fairly similar to those of methionine. Therefore, the pancreas rapidly incorporates this unphysiological compound into the digestive enzymes it produces. The liver concentrates an even larger amount of this compound, but calculated per unit weight the concentration of radioactivity is higher in the pancreas than in any other organ (14). In order to stimulate the pancreatic uptake of radioactivity several measures have been advocated. Intravenous injection of pancreozymin (3), ingestion of a high-protein meal and glutamic acid (11), administration of morphine to close the sphincter of Oddi in order to prevent the secretion of labelled enzymes into the intestine (8) and intravenous glucose (12) have been used.

The clinical use of ^{75}Se-selenomethionine for visualization of the pancreas was first reported in a small number of patients by Blau et al. (3) but more extensive studies have since been published (9–12). These studies have shown that the introduction of pancreatic scanning has brought a promising advance in the difficult diagnosis of pancreatic disease, especially carcinoma of the pancreas. The definite value of pancreatic scanning is still uncertain, and a considerable number of wrong scanning diagnoses in cases where the scan is interpreted as abnormal have been reported (9). This has made us pay special attention to analyzing the reason for obtaining pathological scans in the absence of pancreatic disease.

MATERIAL AND METHODS

Equipment

A Picker Magnascanner III with 3 2 inch sodium iodide crystal and 19-hole focusing lead collimator was used. A photoscan and color scan were obtained. In addition the information was transferred to magnetic tape. The recording instrument was Memograph (Lyrec, Copenhagen, Denmark) equipped with display unit for obtaining polaroid pictures with different background subtraction.

Technique

After an overnight fast the patient was given high-protein meal containing little fat and carbohydrate, as described by Sodee (11). N glutamic acid hydrochloride or pancreozymin was given. One to two hours later 250 μCi of ^{75}Se-selenomethionine (The Radiochemical Centre, Amersham, England) was injected intravenously. About 30 min after the injection scanning was started. The patient was first scanned in the anteroposterior direction for distribution f radioactivity in both the liver and the pancreas. The collimated detector was then angled 15 towards the head of the patient in order to avoid the often obscuring effect of the liver partly shadowing the pancreas. With the detector in this position only the pancreatic area was

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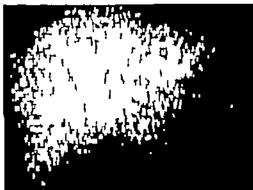


Fig. 5 Pancreatic scan from patient with fist-size carcinoma in the body and tail of the pancreas. Only the head of the pancreas has taken up some radioactivity



Fig. 6. Pancreatic scan interpreted as normal. At operation 2 cm benign insuloma was found in the tail of the pancreas.

liver as in Fig. 3 This was avoided when scanning was performed with the detector in the angled position (Fig. 4) Another factor that often interfered with the interpretation of the scan was the secretion of radioactivity into the duodenum. This is also demonstrated in Fig. 3 where the right border of the head of the pancreas was slightly blurred. In this figure some radioactivity secreted into the upper jejunum can also be seen. The poor visualization of the pancreas in Fig. 5 was due to a fist-size infiltrating carcinoma in the body and the tail of the gland. There was no radioactivity in the intestine and only the head of the pancreas was seen to take up some radioisotope. The scan in Fig. 6 was interpreted as normal, although the possibility that an insuloma, suspected on clinical grounds, was situated in the body was also taken into account. However at operation a _ cm insuloma was found in the tail of the pancreas. In both chronic and acute pancreatitis the gland was poorly visualized because of a diffuse decrease of uptake (Fig. 7). Sometimes the pancreas did not incorporate radioactivity even when an ultimate diagnosis of pancreatic disease was not made (Fig. 8).

An analysis of the accuracy of the scanning diagnosis in 1_2 patients is presented in Table I The overall percentage of correct diagnosis was 75 Of the 69 patients with a normal scan only three had a pancreatic diseas_, and a correct result was thus obtained in 96% In cases diagnosed by scanning as pathological, in only 47% was a correct diagnosis reached. A considerable difference is seen in diagnostic significance between the three subgroups of pathological scan-

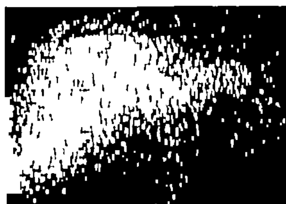


Fig 7 Poor visualization of the pancreas in chronic pancreatitis.



Fig 8 Lack of incorporation of radioactivity in the pancreas of patient with biliary dysfunction after gall bladder operation.

Table I. *Hemodynamic data before and after infusion of Complamin in normal subjects and patients with mitral valvular disease*

			Before		After			
			Mean	S.D	Mean	S.D	*t*	*p*
Oxygen consumption (ml/min)	Normal	10	235	45	226	43	0.96	>0.05
	Mitral valvular disease	1	227	28	226	41	0.08	>0.05
Arteriovenous oxygen difference (ml/l)	Normal	1	36.5	8.2	37.7	5.6	0.19	>0.05
	Mitral valvular disease	12	57.8	14.4	57.1	[illegible]	0.31	>0.05
Cardiac index (l/min/m²)	Normal	10	4.2	1.0	3.7	0.6	0.35	>0.05
	Mitral valvular disease	[illegible]	[illegible]	0.7	2.3	0.4	0.59	>0.05
Pulmonary arteriolar resistance (dynes sec cm⁻⁵)	Normal	9	56	[illegible]	68	3	[illegible]	>0.05
	Mitral valvular disease	11	330	138	317	178	1.68	>0.05
Systemic arteriolar resistance (dynes sec cm⁻⁵)	Normal	8	1015	[illegible]	1128	[illegible]	0.95	>0.05

In the normal persons there was a slight decrease in oxygen consumption and a small increase in arteriovenous oxygen difference during infusion of Complamin®. Both these findings result in a reduction of cardiac output and cardiac index. However, all these changes were insignificant. The mean pressure in the pulmonary artery and wedged position was almost unchanged, and consequently there was a moderate, but insignificant, increase in the pulmonary arteriolar resistance. Furthermore there was an increase in the systemic arteriolar resistance and a reduction in the systolic pressure of the systemic circulation. The heart rate was nearly unchanged, and the stroke volume decreased a little, but insignificantly.

In the group with mitral stenosis during infusion of Complamin® there was almost no change of oxygen consumption, arteriovenous oxygen difference, cardiac index, pressure in wedged position and pulmonary artery, pulmonary arteriolar resistance, heart rate or stroke volume. There was, however, a slight but insignificant fall in the systolic arterial pressure during the infusion. As mentioned, two patients with mitral stenosis had arterial hypotension during the procedure. The infusion was stopped, and these patients were excluded from the material. On statistical evaluation of the material as a single group, the reduction in systolic arterial pressure was insignificant. Systemic arteriolar resistance was only available in four patients, because the systolic systemic

Table II. *Hemodynamic data before and after infusion of Complamin® in normal subjects and in patients with mitral valvular disease*

			Before		After			
			Mean	S.D	Mean	S.D	*t*	*p*
Pulmonary wedge (left atrial) pressure (mean, mm Hg)	Normal	11	6	3	6		0.34	>0.05
	Mitral valvular disease	1	20	8	20	8	0.40	0.05
Pulmonary arterial pressure (mean, mm Hg)	Normal	11	10	2	11	3	0.81	0.05
	Mitral valvular disease	11	35	13	34	17	0.43	0.05
Systolic systemic pressure (mm Hg)	Normal	9	117	4	11	4	[illegible]	0.05
	Mitral valvular disease	1	117	4	110	18	1.68	0.05
Heart rate (beats/min)	Normal	11	6	15	73	15	[illegible]	0.05
	Mitral valvular disease	1	[illegible]	11	83	13	0.54	0.05
Stroke volume (ml)	Normal	9	86	14	79	4	0.91	0.05
	Mitral valvular disease	1	51	15	51	15	0.18	0.05

pressure was measured in the left ventricle in the remaining cases. There were too few patients for statistical evaluation, but on an average the systemic arteriolar resistance decreased from 1,592 to 1,570 dynes sec cm⁻⁵

Infusion of Complamin® produced a cutaneous flush in all patients. Otherwise there were no side effects.

DISCUSSION

No significant changes were recorded in any of the ten hemodynamic parameters during Complamin® infusion, either in the normal group or in the patients with mitral disease. In the group with mitral stenosis there was a small decrease in the systolic pressure in the systemic circulation. In the normal group there was a small decrease in oxygen consumption and a slight increase in arteriovenous oxygen difference. Consequently the cardiac index was a little reduced. There was a trend to an increase of the pulmonary arteriolar resistance and a decrease of the systolic pressure in the systemic circulation.

These findings contrast with the observations of Bachmann (1). With heart catheterization and dye dilution technique he examined two normal persons, one with mitral stenosis and one with operated mitral stenosis. After intravenous infusion of 300 mg Complamin® he found a small increase in heart rate and a great increase in cardiac index and stroke volume. There was no significant change in the arterial pressure, but the mean arterial pulmonary pressure rose from 18 to 26 mm Hg on average. In the operated patient this pressure increased from 24 to 43 mm Hg.

Zetterquist (8) examined a few patients with cardiac catheterization before and after intramuscular injection of 450 mg Complamin®. At rest the heart rate increased, and the pressure in the systemic and the pulmonary circulation fell. Simultaneously there was a small increase of stroke volume and cardiac output. During work there was a general fall of pressure in the pulmonary artery and the systemic circulation. The cardiac index was on an average 14% smaller after Complamin® than before, whereas the reduction of mean stroke volume was 26%.

In the present study Complamin® gave rise to a small reduction of the cardiac index in the normal group. A similar fall, however, very often occurs, as the persons become gradually adapted to the procedure, unaffected by drugs. Müller (4), in 76 normal subjects during rest, found an average fall of the cardiac index of 4.4% from 4.33 to 4.14 l/min/m². In the present material infusion of Complamin® caused a somewhat greater mean reduction of 11.9% from 4._ to 3.7 l/min/m².

Among the small changes in the present study a trend to reduction of the systemic arterial pressure has been recorded. Two patients with arterial hypotension during infusion of Complamin® were excluded from the material, as the examination was not completed. There is a possibility that this hypotension was caused by Complamin®, but this has not been proved, since arterial hypotension sometimes also occurs in ordinary cardiac catheterizations, uninfluenced by drugs. No increase was recorded in the pressures of the pulmonary artery or in wedged position of the catheter either in the normal subjects or in the cardiac patients. In conclusion, there seems to be no contraindication to the use of Complamin® in patients with mitral stenosis.

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HEREDITARY AMYLOIDOSIS WITH POLYNEUROPATHY

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Abstract. An account, mainly clinical, is given of 18 cases of hereditary amyloidosis with polyneuropathy. Common to all cases were progressive peripheral sensory-motor nervous disturbances starting in the legs. Gastro-intestinal dysfunction with malabsorption occurred frequently as did various symptoms of autonomic nervous disturbances. Characteristic opacities of the corpus vitreum were found. Various ECG changes occurred, indicative of amyloidotic heart involvement, which also may have contributed to congestive cardiac failure in some of the cases. The value of examining biopsy specimens in polarized light is emphasized. Familial cases of this form of amyloidosis have not been reported from Scandinavia before.

In recent years hereditary forms of amyloidosis have aroused an ever increasing interest. From Scandinavia reports have been made of familial heart amyloidosis (10) and of cases similar to familial Mediterranean fever (6). During the past few years some 20 cases of hereditary amyloidosis with polyneuropathy have been diagnosed within the Umeå region. A preliminary report of the first cases has already appeared (1). In 1952 Andrade (2) reported cases of hereditary amyloidosis with polyneuropathy in Portugal. Since then similar reports have appeared from different parts of the world (4 8, 15 17 21 22). One sporadic case of primary amyloidosis with clinically pronounced polyneuropathy has been reported from Finland (20). As far as is known, hereditary amyloidotic polyneuropathy has not previously been reported from Scandinavia.

MATERIAL

The material in this study, chiefly clinical, consists of 18 cases. Six cases, A. 1 A. 6, belong to a family called A. Six cases, B. 1 – B. 6, belong to family B. A father and his son form family C. Four cases, *a–d* are "sporadic". Clinically and histologically however they are identical to the cases in question, and similar symptoms were manifest in some of their deceased relatives. Two brothers of case are said to have had similar symptoms; one cousin of case and the father of case *d* are reported to have had similar trouble. In some cases (A. 3–5 B. 2, 4, 5 and C. 1) clinical data were received from other hospitals.

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METHODS

Biopsies for histological examination were usually taken from the rectal mucous membrane the sural nerve and from the skin distal to the fore part of the lower leg. In some cases (A 5 B 2 and *a*) the diagnosis was confirmed by re-examination of the available autopsy or biopsy material. The staining methods were the van Gieson, methyl-violet and alkaline Congo red. Sections stained with Congo red were examined in polarized light. The histological investigation was carried out by Dr P.-A. Hofer Department of Pathology University of Umeå.

Stool fat content was determined, according to van de Kamer et al. (14), as the mean value of the total fat from three days with ordinary diet. Normal value < 6 g total fat/24 h. The five-hour urinary excretion of D-xylose was determined following an oral 25 g dose. The D-xylose was assessed according to Roe and Rice (19). Normal value > 5 g/5 h.

The concentration of folic acid in the serum was determined with Lactobacillus casei (Dr A. Kallander Institute of Medical Chemistry Uppsala) Normal value 3.1 15 ng/ml, border line value 2.1 3 ng/ml, and low value 0–2.0 ng/ml.

CASE REPORTS

A report of two cases is given below which appear to be typical of the disease. Data concerning all the 18 cases are given in the Tables and are not usually repeated in the following case reports.

Case A. 1

Male born in 1899. As a child the patient had had parotitis without complications. At the age of 70 he had



Fig. 1. Marked muscle wasting especially in the distal parts of the arms (case A. I).

a period of icterus, probably mild infectious hepatitis. In the early 1940's he received hospital treatment for gastric ulcer. In 1963 he underwent an operation for fracture of the left collum femoris. It was then observed that the patient had an enlargement of the heart with transient symptoms of congestive cardiac failure. The ECG revealed atrial fibrillation and bundle branch block. At the age of [illegible]0 he began to have trouble with habitual chilliness of the feet, some years later with prickling, creeping and numbness of the feet. At the same time he had also more or less marked pains in the lower limbs. Later on similar symptoms arose distally in the arms. During the following years up to hospitalisation he noticed increasing paresthesia in the upper and lower limbs. In the course of time he lost [illegible] feeling distally in the extremities. He also noticed progressive weakness and wasting of the muscles. Small skin lesions arose several times on the feet.

Impotence arose when the patient was about [illegible]5 years of age. At that time he also began to have symptoms of gastrointestinal trouble with dyspepsia, distension and alternating diarrhoea and obstipation. The year before hospitalisation sphincter disorders developed.

The patient was admitted to the Department of Medicine in Lund in 196[illegible]. On admission physical examination proved him to be a chronically sick man and moderately reduced in weight. He had slight dyspnoea and his lips were cyanotic. There was no oedema in the legs at that time. The skin of the feet was remarkably shiny and thin and had a bluish-red discoloration. The skin of the hands was also atrophic with two small ulcerations on the back of each hand. Otherwise there were no infiltrations or local changes in the skin. The cranial nerves were normal. The pupils were round, of the same size, and reacted normally to light and accommodation. The eye grounds were normal and the media clear.

The patient had symmetric wasting of the muscles and weakness in all extremities. This was pronounced in the feet and lower legs, and also in the hands and forearms (Figs. 1 and 2). More moderate wasting was found proximally in the extremities and in the muscles of the trunk. He was unable to rise from a reclining position without help. He could stand up and walk only with support and then with a marked foot drop.

No tendon reflexes could be elicited in the upper or lower limbs. Plantar stimulation produced no reaction on either side. Fasciculations were observed in the muscles of the upper and lower parts of the arms and in the lower parts of the legs. Moderate dysmetria was found in all the extremities.

Sensibility examination revealed symmetrically reduced perception for all qualities in the extremities, chiefly pronounced distally. The sense of temperature and pain was absent to the height of the knees and elbows respectively. There was no perception of touch in the hands and feet. The sense of vibration and position did not exist

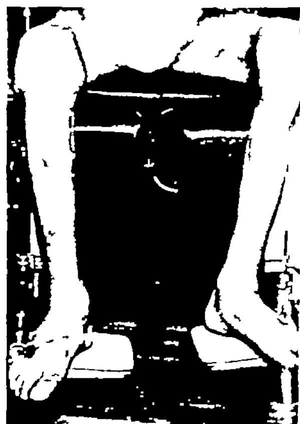


Fig. 2. Muscle wasting and trophic skin in the legs. Same patient as in Fig. 1.

distally to the ankles and wrists respectively. Sensibility was successively better in proximal direction.

Examination of the heart revealed general enlargement and apical systolic murmur. There was atrial fibrillation. The blood pressure was 160/80.

A thorough investigation was made to obtain an aetiological diagnosis of this case with polyneuropathy. There was no history of exposure to neurotoxins. No signs of chronic infection or inflammatory process could be found, nor any cancerous disease.

A number of laboratory tests were carried out which did not show any specific deviations. The ESR was about 20 mm/hour, the haemoglobin 14.5 g per 100 ml. The number of white blood corpuscles was 6,600 with normal differential count. The smear of sternal marrow was normal. No abnormalities of the plasma cells were seen. The protein electrophoresis of the serum and urine was normal.

Histological investigation of biopsy from the gastrocnemius muscle revealed obvious muscular atrophy of the neural type, but did not substantiate an aetiological diagnosis. Primary amyloidosis was suspected, particularly as information had been received that three cousins of the patient had symptoms of polyneuropathy. Moreover two of them were reported to have impaired vision because of vitreous opacities. Further biopsies from the patient for amyloidosis—from the rectum, tongue, gingiva, liver, skin and kidney—yielded negative results. These histological investigations were carried out with ordinary light microscopy. The patient was discharged without any explanation of his progressive polyneuropathy having been found.

One of the aforementioned cousins, case A. 2, was later admitted to the Department of Medicine in Umeå. A nerve biopsy in her case revealed amyloidosis. When case A. 1 was again admitted to the Department at a later date, a nerve biopsy proved the presence of amyloidosis in him as well. A later check-up of previous biopsies investigated in polarized light revealed amyloidosis also in other tissues.

The patient's symptoms have since progressed slowly and he is now practically bedridden.

Case B 1

Female born in 1906. She had had pneumonia in 1936 and was operated on in 1963 for benign ovarian cyst, and in 1964 for cholelithiasis.

The actual symptoms first appeared in 1962. She had piercing, burning pains in the feet and calves. Some months later she experienced cushion-like feelings under her feet when walking. She also noticed a difficulty in feeling the temperature of her bath-water with her feet. Repeated investigations revealed reduced sensibility distally in the legs, a certain wasting of the lower leg muscles and also weak achilles reflexes. Symptoms arose in the hands and arms. There was subsequently a rather rapid progress of the symptoms in all limbs with increasing weakness of muscles and reduction of sensibility. A marked and progressive disorder of intestinal function developed. She experienced frequent attacks of diarrhoea. The sphincter function of the bowel was impaired.

In 1966 there was a relatively sudden disturbance of vision in the right eye. She complained of dark, moving threads in the field of vision.

The patient was admitted to the Department of Medicine in Umeå in 1966. Physical examination proved the patient to be a chronically sick, almost cachectic woman. (During the past 3 years she had lost weight to the extent of 20 kg.) The skin was dry and of poor turgor. She had small, trophic ulcers on her fingers and feet. Physical examination of the heart and lungs revealed nothing worthy of note. In reclining position the blood pressure was 110/80. The liver and spleen could not be palpated. There was no adenopathy.

The cranial nerves were normal. There was no impairment as regards the size of the pupils. The pupil reflexes were similar and normal. There was a general and symmetric muscle wasting, most markedly confined to the distal muscles. The patient could walk only with assistance and then demonstrated a bilateral foot drop.

Sensibility examination revealed analgesia of the legs and trunk up to the umbilicus. There was no sense of temperature or touch up to the groin. A weak perception of vibration was found in the crista iliaca, but not distally beyond. Extremely reduced sensibility occurred in the arms up to the clavicles, particularly to pain, temperature and touch. There was no sensibility at all in the hands.

There was general tendon areflexia.

Examination of the eyes revealed normal condition in the anterior segments and lenses. In the right eye there were floating, band-like opacities in the anterior part of the corpus vitreum. In the posterior part, adjacent to the retina, there were moderate, reticular opacities. The vitreum of the left eye appeared to be normal, and similarly the eye grounds on both sides.

The ESR was 14 mm. The haemoglobin was 13 g per 100 ml. The white blood corpuscles numbered 4 400, and the differential count was normal. Laboratory investigation revealed signs of malabsorption.

Electromyographic examination showed pictures in the feet indicating total denervation. In the anterior tibial muscle pictures were found that represented pronounced denervation.

As a result of previous experience with cases A 1 2, amyloidosis was suspected. At first a rectal biopsy proved normal, but the diagnosis was confirmed by examination of a biopsy from the sural nerve.

RESULTS

The composition of the material, the age at onset of the first symptoms and the initial symptoms are given in Table I. The one thing these reported cases had in common was the more or less rapidly progressive polyneuropathy (Table II).

The peripheral neuropathy has been graded clinically as follows:

1. Advanced (+ + +), where there was a total lack of sensibility distally in the limbs, and pronounced muscle wasting.

Table I. *Data on 18 cases of hereditary amyloidosis with polyneuropathy*

N = neuropathy of feet. O = opacities of corpus vitreum. I = impotence. G = gastrointestinal disturbances. C = cardiac arrythmia.

Case	Sex	Age at onset of symptoms	First symptoms	Age at examination	Age at death
A. 1	♂	50	N	66	
A. 2	♀	53	O	63	
A. 3	♂	40	O	50	54
A. 4	♂	35	N	48	57
A. 5	♂	46	N	70	77
A 6	♂	56	N	61	
B 1	♀	56	N	60	61
B 2	♀	63	N	68	71
B 3	♂	40	I	43	
B 4	♂	57	N	60	65
B 5	♀	57	N G	64	67
B 6	♂	51	N	57	
C. 1	♂	56	N	63	70
C. 2	♂	29	N	34	
a	♂	50	N G	59	68
b	♂	49	N G	51	53
	♂	55	N	58	
d	♀	54	N C	63	

2. Moderate (+ +) where there was no sensibility or greatly reduced superficial sensibility distally in the limbs, and certain degree of muscle wasting and weakness.

3. Slight (+), if there were symptoms of neuropathy such as paresthesia, chilliness, numbness and signs of disordered sensibility to temperature and pain.

Gastrointestinal disorder has been graded as follows:

1. Advanced (+ + +), where there were constant attacks of diarrhoea with signs of malabsorption.

2. Moderate (+ +) denotes attacks of diarrhoea alternating with periods of obstipation or normal function.

3. Slight gastrointestinal trouble (+) denotes symptoms such as constipation, meteorism, distention and borborygmus.

Peripheral neuropathy

In all cases the symptoms of peripheral neuropathy began distally in the lower extremities. After varying periods of time the symptoms also occurred in the upper extremities. The initial symptoms were usually irritative *sensory* phenomena such as paresthesia with numbness, prickling and burning. Attacks of piercing, stabbing pain in the limbs occurred. Some patients also had more prolonged pain in the muscles, which was described as ache after exercise and feeling of cramp in the calves. An early phenomenon appeared to be impaired sense of temperature, with cold or burning feelings in the feet. The sensory impairment progressed in a proximal direction and affected all qualities. The most marked disorder was that concerning the perception of temperature and pain, somewhat less in the case of the perception of touch and of the proprioceptive sensations.

Weakness of the extensor muscles of the toes was found to be an early symptom of lower *motor*

Table II. *Neuropathy and gastrointestinal disturbances in 18 cases of hereditary amyloidosis with polyneuropathy*

Case	Neuropathy[a] Legs	Neuropathy[a] Arms	Gastrointestinal disturbances[a]	Impotence	Incontinence Rectum	Incontinence Urinary bladder
A. 1	− + −	+ +	+ +	+	+	
A. 2	− + −	− + +	+			+
A. 3	+ + +	+ +	+	+	+	
A. 4	+ + −	+ +	+	+		
A 5	+ −	+ +	+			
A. 6	+ +	+	+ +	+	+	
B 1	+ + +	+ +	+ + +		+	
B 2	+ + +	+ +	+ + +		+	
B 3	+ +	+	+ + +	+	+	+
B. 4	+ +	+ +	+ + +	+	+	+
B 5	+ + +	+ +	+ + +		+	
B 6	+ +	+	+ + +	+	+	
C 1	+ + +	+ −	+			
C. 2	+ +	+	+ + +	+		
	+ + +	+ + +	+ + +	+	+	+
b	+ + +	+ + +	+ + +	+	+	+
	+ +	−	+	+		
d	+ +	+ +	+ +			

+ + + = advanced, + + = moderate, + = slight. [a] + = early onset.

Table III. *Data on cases of hereditary amyloidosis with polyneuropathy*

Case	Gastro-intestinal disturbances[a]	Weight loss[b]	Urinary D-xylose (g/5 h)	Faecal fat (g/24 h)	Serum folic acid (ng/ml)	Serum creatinine (mg/100 ml)	Proteinuria (Albustix)
A. 1	+ +	+	2.4	3.3	1.9	0 9	+
A. 2	+	+	6 6	6	2.5	0.7	+
A. 6	+ +	+	6.3	2 6	5.3	0.9	−
B. 1	+ + +	+	1 7	14		1.0	+
B 2	+ + +	+	1.3	3.4	5.7	1 7	+
B 3	+ + +	+	1.0	11	2.7	1 4	+
B 6	+ + +	+	2.2	9.6	2.5	0.9	−
C: 2	+ +	+	5.1	5.7	2.7	0.8	−
	+ + +	+	3.1	41	2.4	1 1	+
b	+ + +	+	0.5	61		1 1	−
	+		5.2	6	1 2		−
d	+ +	+	1 4	3.9	4.5	0.7	−

+ + + = advanced, + + = moderate, + = slight. [b] + = marked weight loss.

neurone lesion. Later neural atrophy and flaccid pareses progressed in a proximal direction. A wide-gauged, tottering gait with foot drop developed. Several patients were finally unable to walk or stand without support. Muscle wasting in the upper limbs subsequently ensued and was most pronounced in the hands. Finally wasting appeared also in the muscles of the pelvis and shoulders and similarly in the trunk. The most advanced cases were seriously disabled and finally bedridden.

In some cases there was in part a lateral difference, particularly in the beginning. Usually however the changes were symmetrically scattered.

Four patients (cases A. 1–2, B 2–3) had rather pronounced *fasciculations* in the muscles of both arms and legs. In the case of B 2 this primarily had caused the suspicion of amyotrophic lateral sclerosis. Case B 3 had fasciculations and partly atrophy of the tongue, indicative of *bulbar* involvement.

Nine patients were subjected to neurophysiological examination (cases A. 6, B 1–3 6 C. 2, and *b*–*d*). In cases with clinically conspicuous symptoms the EMG yielded a picture representative of pronounced denervation. The conduction velocity in the peripheral nerves was greatly reduced. In some cases there was a total lack of function. (The neurophysiological examinations were carried out at the Laboratory of Clinical Neurophysiology University of Umeå. Head. Dr S. Blom.)

Examination of the liquor cerebrospinalis in nine cases showed a slight increase of the total protein in three cases (A. 1 B 1 and *a*) and a more pronounced increase in case *b* The paper electrophoresis on liquor did not reveal any specific changes (cases A. 6 B 1–2, C: 2, *c* and *d*) In none of the cases was there any pleocytosis.

The autonomic nervous system

Various symptoms of disorder in the autonomic nervous system were recorded. Sphincter disturbance was found in several cases (Table II) Impotence occurred and appears to have been relatively early Case B 6 observed failure of ejaculation prior to impotence. The various symptoms of disordered gastrointestinal function, e.g. impaired motility meteorism, distention, are probably conditioned by disturbed function of the autonomic nerves, caused by amyloidotic degeneration.

Postural hypotension and disturbance of sweating were other signs indicating autonomic nervous dysfunction. Trophic skin ulcers appeared. In many cases the skin was dry thin and shiny

The gastrointestinal tract

Symptoms of gastrointestinal disturbances occurred frequently (Table II). Nine patients (cases B: 1–6, C: 2, *a* and *b*) had constant diarrhoea. Steatorrhoea was found in seven cases, and the D-xylose absorption test showed low values of urine excretion in eight cases (Table III). Estimation of folic acid in the serum of ten cases showed

Table IV *Data concerning the cardiovascular system in cases of hereditary amyloidosis with polyneuropathy*

Case	Sex	Age at examination	Symptoms	Heart volume (ml/m²)	Blood pressure supine	Electrocardiogram
A 1	♂	66	Dyspnoea, oedema	825	160/80	Atrial flutter VPB, ST T segment depression, marked left axis deviation
A 2	♀	63		510	150/95	Marked left axis deviation
A 6	♂	61		370	140/85	Normal
B 1	♀	60			110/80	VPB left axis deviation
B 2	♀	68	Dyspnoea	450	145/80	Low voltage ST T segment depression marked left axis deviation
B 3	♂	43		530	125/80	A V block I
B 6		57		340	120/85	Normal
C 2	♂	34		310	110/75	Normal
a	♂	59	Dyspnoea, oedema	770	115/85	VPB, A V block II right bundle branch block, left axis deviation
b		51		390	90/60	ST T segment depression, left axis deviation
	♂	58	Dyspnoea	490	145/105	VPB, A V block I marked left axes deviation
d		63	Stokes–Adams attacks	620	175/100	Internal pacemaker

VPB – entricular premature beats.

values below 3 ng/ml in seven, and below 2 ng ml in two of them.

As a more unspecific symptom, reduced weight was pronounced in several cases and appeared relatively early in the course of the disease.

The cardiovascular system

eral patients had cardiovascular disorders (Table IV) Various ECG changes were found, particularly disturbance of conduction and/or ar rythmia. Left axis deviation was common. Case *d* developed Stokes-Adams attacks and has now an internal pacemaker Low blood pressure was found in several cases some of whom had postural hypotension. Three cases had heart enlargement and signs of congestive failure (cases A. 1 *a* and *d*)

Eye symptoms

Of the 18 cases reported, three (A. 2–3 B 1) had pronounced vitreous opacities, which have previously been reported (1). The demonstration of vitreous opacities in case A. (Fig. 3) was of diagnostic importance in these cases. In cases A. 2–3 the opacities appear to have been the first symptoms of the disease. In all three cases the opacities progressed and resulted in total blindness for two of them. Other eye symptoms were ptosis, anisocoria, irregular pupils and sluggish pupillary re flexes. There were typical Argyll–Robertson pupils in case *a*. In that case the Wasserman reaction was negative in the blood and liquor

Histological examination

Examination by ordinary light microscopy of biopsy specimens of the sural nerve revealed amyloid deposits in three cases (A. 1–2, B 1) Previous examinations of several other biopsies from these patients did not reveal amyloid substance. By using alkaline Congo red and examining in polarized light, a better demonstration of amyloid was obtained. The diagnosis was established ante-mortem in nine cases. In two (C: 1 and *b*) the diagnosis was obtained at autopsy In cases A. 5 B 2 and *a* the amyloid was demonstrated by re-examination, with polarized light, of the available autopsy or biopsy material (Table V) Case A. 5 had had polypi in the urinary bladder during his last years. Signs and symptoms of polyneuropathy had arisen much earlier but only the bladder was histologically examined. In case B 2 amyotrophic lateral sclerosis had been suspected, and only the spinal cord was examined. Four cases (A. 3–4 B 4–5) were not proved histologically The clinical data, however reliably verified the diagnosis, e.g. the demonstration of vitreous opacities in case A. 3

The amyloid tissue in these cases had a pericol lagenic (11 18) distribution. A more detailed report of the histopathological findings will appear later (12).

Genetic studies

Figs. 4 and 5 represent preliminary pedigrees of families A and B.

Family A is descended from Finnish immigrants who settled in North Sweden in the mid 17th century (9). Hitherto it has been impossible to establish a relationship between the two families. The sporadic cases, *a–d* may have a connection with *family A* or B.

It is of interest to note that some other patients, especially in family A, had signs and symptoms of polyneuropathy (Figs. 4 and 5). Histological examinations, however were negative for amyloid.

DISCUSSION

The clinical signs for all patients reported on were rather identical as regards polyneuropathy In all cases the initial symptoms from the peripheral somatic nerves appeared in the lower extremities. In this respect they coincide with the Portuguese cases (2).

Age of onset has varied from 29 to 63 years. The average age of onset for men was about 45 and for women about ten years later Data as regards age when the first symptoms occur are at times somewhat uncertain. In some cases earlier symptoms appeared in more than one system of organs. The onset of the disease in these cases



Fig. 3. T the right of the figure the anterior part of the corpus itreum is seen to be clouded by opacities. Slit lamp examination of case A. 2. (From Andersson, R. & Kassman, T Vitreous opacities in primary familial amyloidosis. Acta ophthal. (Kbh.) 46 441, 1968. Reproduced with permission.)

Table V *Histological examinations confirming the diagnosis in cases of hereditary amyloidosis with polyneuropathy*

Case	Biopsy specimen ante-mortem	Post-mortem examination
A 1	Nerve, skin, rectum, gingiva	
A 2	Nerve skin	
A 3	Not examined	
A 4	Not examined	
A 5		Urinary bladder[a]
A 6	Rectum	
B 1	Nerve, skin, rectum	
B 2		Spinal cord
B 3	Skin, rectum	
B 4	Not examined	
B 5	Not examined	
B 6	Nerve, skin	
C 1		Kidney
C 2	Nerve, skin, rectum	
		Skin, muscle
b		Various tissues
	Nerve, skin rectum	
d	Rectum	

Re-examination with alkaline Congo red and polarized ligh

appears, on an average, to be somewhat later than among cases reported from other countries.

The neuropathy which began in the legs, has sooner or later involved the upper limbs and likewise the trunk. In some cases the neuropathy progressed rather slowly This is true particularly among the patients belonging to family A. As regards the patients in family B all of whom had much more pronounced gastrointestinal dysfunction, the neuropathy progressed markedly quicker just as the whole disease had a more rapid course in these cases. This difference in different families has not been previously described.

In all patients the neuropathy increased to a more or less pronounced invalidity resulting in an all too early incapacitation. Invalidity was worst among patients who also had marked diarrhoea and malabsorption.

Heart amyloidosis with congestive failure is said to occur frequently in sporadic cases of primary systemic amyloidosis (13). Amyloid deposition in the heart is also common among elderly people —senile cardiac amyloidosis (23). Of the heredofamilial amyloidoses the cardiopathic group, described from a Danish family (10), has been isolated as a special clinical entity in view of its particularly conspicuous heart involvement. On the other hand congestive heart failure is said to

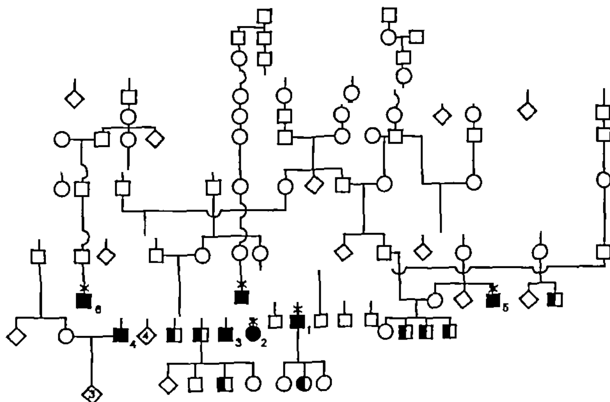


Fig. 4. Pedigree of family A. Numerals to the right of symbols refer to case number (see Material). Diamonds designate sibs of both sexes; the number within the symbol refers to the number of sibs when known. Symbols: □, male; ○ female; ■, affected; amyloidosis proved by histological examination; ◧ signs and/or symptoms of polyneuropathy. *a*, case reported in 1926.

very rare in the neuropathic form of familial amyloidosis (3, 7). Various ECG changes are, on the contrary, common. Of the 18 cases reported here, three had serious heart symptoms earlier in the course of the disease. Two of them, cases A. 1 and *a* had pronounced congestive failure. Case *d* had complete A–V block and Stokes–Adams attacks. She had also a marked enlargement of the heart. The father of case A. 1 died suddenly at the age of 45 with the symptoms of heart insufficiency.

Genealogical studies of sporadic cases who have died of primary amyloidosis at the Department of Medicine in Umeå revealed that one case, minutely reported in 1926 (24), belonged to family A (*a* in Fig. 4). The patient died at the age of 34 because of heart amyloidosis with congestive failure. Symptoms of peripheral neuropathy do not seem to have been manifest.

This case, together with the other aforementioned cases, denotes a higher frequency of congestive heart failure among these familial cases of amyloid polyneuropathy than among those previously reported.

The diagnosis was established ante-mortem in our three first cases (cases A. 1–2, B. 1). Clinically the demonstration of typical vitreous opacities strongly supported the diagnosis. Since the first report (15) of vitreous opacities in cases like these, its occurrence has been confirmed by others (8, 16, 21, 22). Examination later of the autopsy material from case B. 1 showed that the opacities consisted of amyloid substance (12).

Of 12 patients examined for malabsorption seven had steatorrhoea. Clinically case B. 2 too had signs of malabsorption. The normal value of stool fat content in that case may be due to the low administration of fat. The patient had a pronounced intolerance to food.

The D-xylose absorption test gave low values in eight of the 12 cases. This may reflect a real resorption defect localized to the intestinal wall. It may be pointed out, however, that a neurogenic disorder in the digestive canal may influence the

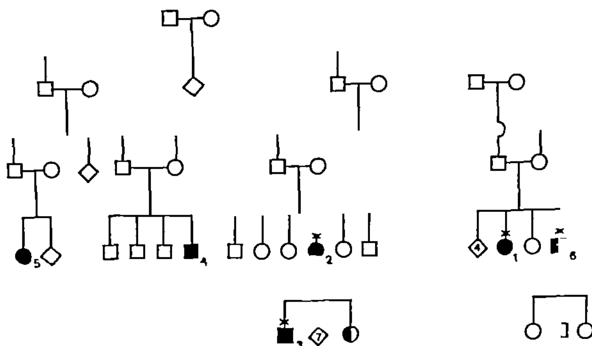


Fig. 5. Pedigree of family B. Numerals and symbols as in Fig. 4

peroral resorption test. In these cases disturbed gastrointestinal motility such as slowly emptying of the stomach, seems to be common.

Neurogenic dysfunction of the urinary tract may similarly contribute to erroneous values in tests demanding urine accumulation.

It seems to be characteristic of this form of systemic amyloidosis that grave kidney insufficiency seldom occurs. Proteinuria was found in six out of 12 cases, but the creatinine in the serum was normal in *four* of them, and only *slightly* increased in two cases (Table III). Uraemia was, however, a contributory cause of death in one case (C: 1).

As in other reports, the affection of the liver was insignificant. Neither clinical nor laboratory manifestations of liver involvement occurred in the present cases. Investigation did not reveal any chronic inflammation or neoplastic disease. Neither could any signs of myelomatosis be demonstrated. In no case did the paper electrophoresis of the serum disclose any peak of alpha$_2$-globulin, which has been reported in some cases (21).

Primary familial amyloidosis with polyneuropathy is reported to have an autosomal dominant mode of inheritance (2, 5, 8, 17). This may be so in these cases too. Genetic studies hitherto are only preliminary. A further ten cases with more or less advanced polyneuropathy but with negative biopsies for amyloid, have been observed, especially in family A. This will be the subject of further investigations.

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Addendum. Since the manuscript was submitted for publication further cases with signs and symptoms of this disease have been diagnosed within the region. Amyloidosis has been proved by histological examination of biopsy specimens in 13 more patients. Some of them belong to family B. Other cases, familial or sporadic, have hitherto no proved connection with the earlier known families.

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ATYPICAL CASE HISTORIES AND ELECTROCARDIOGRAMS IN MYOCARDIAL INFARCTIONS

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Abstract. The case histories and ECGs of 215 patients with a diagnosis of recent myocardial infarction have been examined with special reference to atypical findings. One hundred and seventy-six had their first infarction, while 39 had had at least one myocardial infarction previously. Sixty-eight per cent had typical and thirty-two per cent atypical ECGs according to accepted criteria. The different non-diagnostic ECG patterns are summarized, showing that 40% had ST-T changes as the main change, 17% unchanged Q-wave pattern from previously known myocardial infarctions, and 1% the R/S pattern of Perloff (19). It is suggested that patients with this R/S pattern (in the absence of pitfalls of Perloff) should be considered to have strictly posterior myocardial infarctions. The inclusion of these patients would increase the diagnostic score from 68% to 75% in the present study. No patient had a completely normal ECG. Discussion of some simple, general ECG principles shows that one can never expect to diagnose more than a certain number of recent myocardial infarctions in the ECG. The most common reasons for the ECG being non-diagnostic of myocardial infarction are summarized. It is also stressed that Q-wave patterns are not always diagnostic of myocardial infarctions, as they may also be seen in other more rare forms of heart disease, probably most often in primary myocardial disease. Ten per cent had atypical case histories, and the different presenting symptoms are summarized. Thorough examination of patients with such symptoms with special reference to possible myocardial infarction as the underlying cause may disclose a certain number of recent myocardial infarctions which would otherwise have been overlooked.

There still seems to be much controversy about the electrocardiographic criteria for a diagnosis of myocardial infarction. Autopsy studies (5, 12, 13, 23, 26, 30, 31) seem to show that a considerable number of infarctions are overlooked in clinical practice.

Singly or in combination with other tests, examination of the ECG offers the best clues to the diagnosis of recent myocardial infarction. It has therefore been our purpose to examine the case histories and especially ECGs in a group of patients with a diagnosis of recent myocardial infarction in order to find possible reasons for this underestimation.

MATERIAL AND METHODS

The material comprises all patients with a diagnosis of recent myocardial infarction during a period of two years on the proviso of a case history of less than 48 hours duration before admission to the hospital. The diagnoses were based on case histories, ECGs and SGOT values. The ECGs were ordinary 12-lead ECGs taken with Cardiopan ECG analyzer. Pathological Q-waves in lead I, aVL, V_1–V_6 have been taken as signs of anterior myocardial infarction. Q-waves in lead II, III, aVF have pointed to a "posterior" myocardial infarction, while ECGs without pathological Q-waves have been called atypical. In all (except a few who died shortly after arrival and in whom the diagnosis was confirmed at autopsy) at least one transaminase value exceeded 40 Sigma units. Patients with atypical ECGs had typical case histories and SGOT values as stated above.

All the patients have been especially asked if they had had any chest or upper abdominal pain during the first part of their illness. Case histories with pain have been called typical case histories, while case histories without pain have been called atypical. All cases with atypical case histories had typical ECGs.

In all who lived long enough, ECGs were taken and SGOT was estimated daily for the first three days of the stay. Beside this, ECGs were taken just before the patients left the hospital. Additional ECGs in most patients were taken during the stay. In addition, the diagnoses were checked by thorough daily examinations, leucocyte counts, ESR, temperature curves, and serum-LDH estimations.

The ECGs were examined by two of the authors (J. E. and N. A.). To distinguish between primary and secondary ST-T changes the ventricular gradient approach (10) was used. Elsewhere ordinary scalar principles have been followed (14).

No patient has been included more than once. If a patient had more than one infarction during the period, only the first episode has been included.

Table I. *Clinical data*

	Group I	Group II
	Patients with unequivocal signs of recent myocardial infarction in the ECG ("Q-wave infarction")	Patients with equivocal signs of recent myocardial infarction in the ECG
	145 pats. (68 %)	70 pats. (32 %)
AI	68 pats.	
PI	77 pats.	
FMI	118 pats.	58 pats.
PMI	27 pats.	12 pats.
$SGOT_{max}$	155 Sigma units	125 Sigma units
MR	33/145 (23 %)	8/70 (11 %)

AI = anterior myocardial infarction. PI = posterior myocardial infarction. FMI = first myocardial infarction. PMI = previous myocardial infarction. $SGOT_{max}$ = average maximal SGOT value for the whole group. MR = mortality rate.

RESULTS

The material comprises 215 patients, 57 females and 158 males. Thirty-nine had previously suffered from at least one myocardial infarction, while 176 had their first infarction during the trial. In 145 (68 %) there were unequivocal signs of recent myocardial infarction in the ECG: sixty-eight were judged to be anterior myocardial infarctions (anteroseptal, anterolateral, strictly anterior and apical myocardial infarctions), and seventy-seven to be posterior myocardial infarctions (diaphragmatic and diaphragmaticolateral myocardial infarctions). Twenty-seven of the 145 patients with unequivocal signs of myocardial infarction had previously had at least one myocardial infarction localized (electrocardiographically) in a different part of the left ventricle. One-hundred-and-eighteen of the 145 had their first myocardial infarction. From Table I it is seen that the average maximal SGOT value in this group was 155 Sigma units and the mortality rate 23 %. These 145 patients (group I) have not been examined further.

Seventy patients (32 %) had equivocal signs of recent myocardial infarction, and these patients have been examined more thoroughly. The average SGOT (max.) value in this group was slightly

Table II. *ECG findings and clinical data in patients without significant Q-waves*

Main ECG change	No of pats.	Deaths	Average age (range)	SGOT max. average (range)	Relative heart volume (range)	Treated with digitalis
Old infarction pattern	12	3	67.7 (61–83)	135 (66–330)	530 ml (415–680)	11
RBBB	2	1	72.5 (66–79)	127 (83–171)	490	1
LBBB	2	0	78.5 (76–81)	292 (134–450)	450 (440–465)	2
LVH	5	1	75.0 (68–81)	93 (55–127)	580 (480–695)	3
ST T changes (primary)	26	0	61.2 (43–81)	140 (52–700)	475 (335–840)	8
R/S ratio > 1 in V	11	0	56.8 (46–68)	193 (100–350)	440 (285–680)	3
R/S ratio < 1 in V (not in V_1)	4	2	60.3 (51–70)	111 (45–182)	465 (410–520)	
Insignificant Q-waves	4	0	66.5 (53–80)	123 (48–270)	440 (360–590)	
Rudimentary R-waves in V_1–V	2		56.0 (54–58)	(*)	—	
Low voltage	1	0	84	100	545	1
Agonal ECG	1	1	66	(*)	—	1
Normal ECG	0	0				
Total	70	8	64.0 (43–84)	135 (48–700)	480 (285–840)	30

RBBB = right bundle branch block. LBBB = left bundle branch block. LVH = left ventricular hypertrophy. "Old infarction pattern" = typical Q-wave changes of infarction, unchanged from changes seen in earlier myocardial infarction.
* Died before SGOT had been taken (autopsy showed fresh myocardial infarction).
Heart volume (ml/m²) measured in all except the eight dead patients and two very ill, old patients.

Table III. *Combination of ECG patterns (in patients with atypical ECG i.e. group II from Table I)*

	Old inf. pattern	RBBB	LBBB	LVH	ST T changes	R/S > 1 in V	R/S > 1 in V	Insignificant Q-waves	Rudiment. R in V V	Low volt.	
Old infarction pattern	4			2	6						12
Right bundle branch block (RBBB)					2						2
Left bundle branch block (LBBB)					2						2
Left ventricular hypertrophy (LVH)				1	4						5
ST T changes (primary)				4	18			3		1	26
R/S ratio > 1 in V and V					8	1		2			11
R/S ratio > 1 in V (but not in V_1)[a]				1	2					1	4
Insignificant Q-waves				2	1		1				4
Rudimentary R-waves in V_1–V	1								1		2
Low voltage					1						1
Agonal ECG											1
											70

R/S ratio > 1 = R-wave in V (or V_2) (in mm) divided by S-wave in V (or V_2) (in mm).
Main ECG changes (from Table II) (vertical column) are presented horizontally. Obvious secondary changes (if any) (horizontal column) are presented vertically.
Example: 26 had primary ST T changes as the main change. Four of these 26 showed signs of left ventricular hypertrophy as well, three had insignificant Q-waves, and one low voltage, while 18 had ST T changes solely. Besides the 26 with ST T changes as the main change, 26 others had primary ST T changes together with another main change.

less than the value in group I (Table I). Twelve had had at least one myocardial infarction previously while 58 had their first infarction. The mortality rate was 11% or about half that of group I. The different ECG patterns in group II have been summarized in Tables II and III. As may be seen no patient had a completely normal ECG. Primary ST T changes were the most common non-diagnostic changes (40%). Thirty of the patients in group II used digitalis, but only eight of the 26 with ST T changes as the main change. The twelve with previously diagnosed myocardial infarction had unchanged ECGs from an earlier stay in hospital. Eleven had an R/S ratio > 1 in V_1 (and V_2), while an additional four had an R/S ratio > 1 in V (but not in V_1). Due to some difficulties in determining the main change and the less important ECG alterations, the two main changes have been summarized in Table III. It appears that 52 had primary ST T changes according to the ventricular gradient approach. This means that one half had ST T changes as the sole abnormality (Table II) while the other half had ST T changes *and* signs of an old infarction, R/S ratios > 1 in V_1–V_2 etc. In group II 13 had signs of heart enlargement (males > 550 ml/m² females > 500 ml/m²), while seven had borderline values (males 500–550 ml/m² females 450–500 ml/m²). Fourteen had signs of left ventricular hypertrophy (14),

Table IV. *Survey of case histories*

Typical case history	195
Atypical case history (without pain)	20 (10%)
Presenting symptom in the atypical case histories	
Pulmonary oedema	7
Collapse	1
Fainting (without any other symptom)	1
Sudden onset of dementia (mentally alert earlier)	3
Dyspnoea	2
Nausea/vomiting	2
Asthenia	1
Acute psychoses	1
Apoplexy	2
	20

mainly those with X-ray signs of heart enlargement.

In Table IV the case histories of all 215 patients are summarized. It is seen that 10% had case histories without pain.

DISCUSSION

Autopsy studies show that a considerable number of myocardial infarctions are overlooked in clinical practice and this concerns both recent and old infarctions. On the basis of this the following main causes for overlooking an infarction in the hospital have to be discussed.

1 The patient is admitted to the hospital with a non-cardiac disease Symptoms of an intercurrent myocardial infarction may either (*a*) be misinterpreted as symptoms of the disease which brought the patient to the hospital or (*b*) be overshadowed by the symptoms of the other disease(s).

2. The symptoms of the infarction are so atypical that suspicion of myocardial infarction does not arise.

3 The patient suffers from heart disease which has been diagnosed previously The symptoms of an infarction (for instance sudden deterioration of heart failure in hypertensive heart disease sudden arrhythmia(s) etc.) are misinterpreted as symptoms of the heart disease from which the patient is already known to suffer

4 A myocardial infarction is suspected, but the ECG signs and or laboratory tests are equivocal. Among the reasons for this are

(*a*) Delay in laboratory tests and or insufficient number of tests.
(*b*) Serial ECGs are omitted and
(*c*) ECG and or laboratory tests are taken too early
(*d*) ECGs taken lege artis do not show infarction patterns.

5 Non-equivocal signs of myocardial infarctions are misinterpreted.

Causes 1 2 and 3 concern the clinical signs and symptoms, while 4 and 5 concern the objective signs of myocardial infarction. Of the latter problems only the ECG problems are dealt with.

The first problem(s) The literature on the symptoms and signs in myocardial infarctions is vast, and reference is made to recent articles on the subject (5 9 18). When a patient is admitted with a recent history of excruciating retrosternal pain, a diagnosis of myocardial infarction immediately springs to mind. Pathy (18), however shows the extreme variability in the presenting symptoms in the elderly with a recent myocardial infarction. In his work on 387 patients above 65 years of age only 20% presented with a "typical case history" i.e a history of chest pain. Asthenia, mental confusion, apoplexy dyspnoea, weakness and peripheral vascular disturbances were far more frequent as presenting symptoms. In our group 10% had an atypical case history (However in several cases the chest pain was far less distressing to the patient than other symptoms, such as dyspnoea, and was often only revealed on clos questioning.) This may possibly be taken as a sign of underestimation of the true frequency of recent infarction in our elderly patients. To see how often a myocardial infarction is disguised as acute mental confusion, apoplexy and so on, it is necessary to make a prospective study in patients with these symptoms.

To diagnose all cases of myocardial infarction a high degree of suspicion is essential especially in the elderly patients and in patients with complicating heart disease. Very much the same conclusions are drawn by Eisner and Schweizer (5) in their autopsy study of 164 patients in whom the clinical diagnoses of myocardial infarction were missed.

The second problem is why the ECG diagnosis of myocardial infarction is missed.

Most authors agree that the ECG is rarely completely normal in myocardial infarction during the acute stage if serial registrations have been used (26, 28 30) Eisner and Schweizer however state that seven of their 164 patients had a completely normal ECG (there are no comments on the number and/or time of registration of the ECGs during the illness (5)) In our group no patient had a completely normal ECG (Tables II and III).

There seems to be fair agreement that the ST T changes are only suggestive of myocardial infarctions. Too many diseases may cause similar ST T changes (4 10 22). Of our 215 patients 26 had primary ST T changes as the sole pathological sign of their infarctions (12%).

ECGs taken during the period from the third to the tenth day of an infarction are said to be temporarily normalized in some cases (11 27).

ECGs taken before and/or later than the third to the tenth day may according to these authors, show changes which are absent during this period. Our groups are not biased in this way because of the selection made by excluding all patients with a case history of more than 48 hours.

Development of pathological Q-waves is the best sign of myocardial necrosis in clinical scalar electrocardiography Pruitt et al. (22) state that typical Q-waves pattern of myocardial infarc tion rarely develops in other diseases. It *may* however be seen in inflammatory degenerative, infiltrative and metastatic diseases of the myocardium, but in their opinion only rarely Mar riott and Menendez (15) are of another opinion, but their documentations seem less convincing. QR, Qr and QS patterns in special leads, there fore, almost always point to a destructive lesion in the myocardium caused by coronary heart disease. Idiopathic muscular subaortic stenosis (20) and chronic cor pulmonale (2.), however are important differential diagnoses in the ECG

With ordinary" ECG criteria the diagnostic score of recent first myocardial infarction in clinical studies varies from 70% to 90% (6, 23) Earlier myocardial infarctions often pose unsur mountable obstacles to the ECG reader in the interpretation of recent changes. This was the fact in 12 of our 39 patients with previous myocardial infarction (or infarctions). This means, however that 27 showed unequivocal signs of recent infarctions with another localisation than before (or 2/3). The same score of diagnostic Q-wave pattern was found among the 176 patients with their first myocardial infarction.

Autopsy studies show an exactness of ECG solely in the diagnosis of myocardial infarction which varies from 20% to 80% (13 .8). Recent works tend to show a low degree of exactness. A score below 40% could be seen in old myocardial infarctions (?6, 30) while a score of 80% was seen in recent infarction, and about 50% where there were combinations of recent and old myocardial infarctions (30).

In another study some 50% of the infarctions found at autopsy were neither diagnosed nor suspected (12).

The correlations between the ECG localisation of an infarction and the real localisation found at autopsy seem to be still worse (13 24).

The low score of the ECG diagnosis in myocardial infarctions may be explained from basic ECG principles:

The electrical window theory" of Wilson is probably incorrect (8, 10 29) As a rule not more than 10% of local potentials in the precordial region arise from the directly underlying myocardium (10). The ECG therefore, must be considered rather as the sum of an infinite number of local potentials from all parts of the heart. Loss of heart muscle in one part makes the resultant electrical force point more or less away from the infarcted area. This will often create Q-waves in one or more of our conventional ECG leads. It has been shown that the subendocardial area does not contribute to the generation of the QRS (3 4 25). However QS patterns may be seen where the infarction is far from transmural (3 4 16).

While earlier concepts have been that 90% of myocardial infarctions deform the earliest 0.04 of the QRS, recent experimental work showed deformation only of middle and terminal parts of the QRS in dogs (1). Another experimental study on dogs points to the following con clusions: a well marked reduction in the R-wave and rS-pattern in V_1–V should leave no doubt as to the presence of infarction (16) In the future, therefore, we may have to examine more thoroughly the later parts of the QRS. Only two of the patients in the present series showed rudimentary r-waves V_1–V_3. Both these patients died, and possibly both would have had an ordinary QS-pattern in V_1–V_3 if they had lived long enough.

Naval et al. (17) have partly used these principles of late QRS changes in their work on scalar ECGs taken with Frank' (7) corrected orthogonal lead system. Their conclusions are as follows: "Diagnostic recognition rates of Q/R-ratios exceed those of Q-amplitude and Q-wave duration with a wide margin 29 per cent would have been missed without measuring the Q/R-ratio. The Q-wave duration was found to be the only abnormality in 5 cases or 2 per cent of the series

The Q-waves amplitude did not independently contribute to diagnostic performance.

Knowledge of the normal spread of the ex citation wave in the left ventricle (25), moreover would lead us to suggest that lesions in special parts of the left ventricle would deform later rather than earlier parts of the QRS.

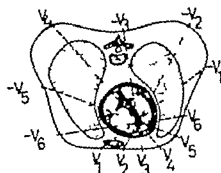


Fig 1 Mirror image points on the chest.

Q/R ratios or conversely R/S ratios have been used by Perloff (19) as a sign of strictly posterior myocardial infarction. He states that an R/S ratio > 1 in V_1 (and/or V_2) points to an infarction in the strict posterior aspects of the left ventricle in patients older than 40 years. He states, however that the same pattern may be seen in pure right ventricular hypertrophy complete right bundle branch block, WPW syndrome type A and in a few adults showing perpendicular or right axis deviation of the QRS in the frontal plane. All these pitfalls were excluded in our 15 patients showing this special R S pattern. It is assumed that these patients had unequivocal signs of strictly posterior myocardial infarction. This is perhaps the most common unequivocal sign of myocardial infarction to be overlooked, according to own experience

The vector approach offers us a better tool when interpreting the scalar ECG When the ECG is considered as the sum of numerous local potentials, we may easily see why a very small lesion may be impossible to discover electrically If it is assumed that there is close correlation between the extension of myocardial infarctions and the SGOT values they cause particularly small infarctions in this group have been seen infrequently and cannot explain the frequency of non-diagnostic changes except in very few cases.

According to the vector approach it appears why two or more infarctions in different parts of the heart may counteract each other electrically The counteraction may indeed be so complete that it is impossible to find any signs of recent infarction or of myocardial infarction at all. This is stressed, for example, by Pruitt et al (..)

In 1. of our 39 patients with previous myocardial infarction it was impossible to detect changes of recent infarction. As the lowest SGOT value in this group was 66 Sigma units, the old infarctions may have caused difficulties in the interpretation of the recent changes.

Through the dipole concept it also appears that pathological Q-waves have their corresponding pathological R-waves on the opposite side of the body (8) (see Fig. 1 which shows the mirror image points *of the common V_1–V_6 leads)* There fore R waves in special leads are as pathological as Q-waves in other leads. As seen above, tall R waves in V_1 (with shallow S-waves) should be regarded as diagnostic of strictly posterior myocardial infarction (19).

Bundle branch block changes the electrical events in such a way that the ordinary ECG criteria cannot be used in the diagnosis of myocardial infarctions.

In summary we have the following main reasons for the ECG being non-diagnostic according to accepted criteria (2, 26):

1 The infarction is too small to be detected in the electrical events of the QRS.

2. The infarction is localized in an area which is electrically inert with reference to the QRS (i.e subendocardium).

3 The ECGs have been taken during the "intermediary phase (third to tenth day)

4 The infarction is localized in an area where (both) early and/or late parts of the QRS are deformed instead of the earliest 0.04 This would cause pathological alteration of Q/R ratios (or R/S ratios) in special leads without necessarily giving rise to pathological Q-waves.

5 The infarction is localized in an area where the ordinary 12-lead ECG does not disclose pathological Q-waves (or Q/R ratios) The counterpart, i.e. pathological R-waves, may be overlooked.

6. Earlier infarctions form obstacles to the interpretation of recent changes.

7 Bundle branch block changes the excitation wave so that ordinary infarction criteria cannot be used.

Combination of two non-diagnostic changes in the ECG (Table III) did not contribute to a better diagnostic performance in the present group. Of the 70 patients with a non-diagnostic ECG however 5. showed primary ST T changes. This probably means that when a non-diagnostic ECG

does not show primary ST T changes, there is little reason to believe that the patient has a recent myocardial infarction. The four patients with bundle branch block all showed primary T-changes in addition to the usual bundle branch block pattern.

CONCLUSIONS

One-third of 215 patients with a recent myocardial infarction had atypical ECGs for various reasons. It is suggested, however, that 15 patients with R/S ratio > 1 in V_1 and/or V_2 had diagnostic changes of strictly posterior myocardial infarction according to Perloff (19). According to Mathur and Kumar (16) it is also suggested that two patients with rudimentary r-waves in V_1–V had unequivocal signs of recent infarction. Apparently this ECG sign should be regarded as diagnostic in the absence of left bundle branch block.

If these 15+. patients are included, the diagnostic score would increase from 145/215 (68%) to 162/215 (75%). In addition, 1. had signs of old myocardial infarction. This means that 174 had unequivocal signs of recent and/or old myocardial necrosis in the ECG The diagnoses in the remaining 20% had to be based solely on other tests. In agreement with other authors, however it appears that a completely normal ECG in myocardial infarction must be rare.

The degree of exactness is probably fictitious according to the above cited pathological anatomic studies (5 12, 13 23 26, 30, 31).

When an infarction is overlooked, the patient may lose the most skilled treatment both during the acute stage and afterwards. Special attention, therefore, should be paid to the atypical ECGs although the immediate prognosis seems to be better than in patients with Q-wave patterns in the ECG A specially favourable outcome was seen in the patients showing ST T changes solely

A high degree of suspicion is necessary especially in elderly patients and patients with complicating heart disease.

It is stressed that atypical case histories, i.e. case histories without pain, are not rare.

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EARLY AMBULATION IN MYOCARDIAL INFARCTION

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Abstract. Two series of patients were compared in an attempt to determine the role of early ambulation in the management of cases of myocardial infarction. The earlier series consisted of 108 patients (80 men, 28 women), the later of 146 (96 men, 50 women). In each series the patients were subdivided into two groups according to the severity of the infarction. In the earlier series the classification was made on the basis of serum enzyme changes, the leucocyte count, hypotension, temperature reaction, and prolonged chest pain. The later series was divided, on the basis of electrocardiographic changes, into intramural and transmural infarctions. In the earlier series the period of complete bed rest at the initial stage was 7 or 14 days and the hospital stay correspondingly 21 or 28 days according to the degree of severity determined at the early stage. In the later series bed rest lasted 3 or 7 days and hospitalization correspondingly 12 or 16 days depending upon whether the myocardial infarction was intramural or transmural. No significant differences were found when comparing the mortality in the series at 7 days and 30 days following the onset of infarction. The results are in favour of early ambulation and suggest that the appropriate length of stay may be shorter than generally stated in the literature.

The effect of bed rest on the prognosis of patients with myocardial infarction is still an open question. It has been customary to give these patients complete bed rest for 4–6 weeks. This length of bed rest was evidently based on studies which showed that 6–8 weeks are required for the myocardial necrosis to be transformed into firm scar tissue (7 10). Since the demonstration by Levine and Lown (8) that the prognosis even improves if the patient is allowed to sit up in the early stage, there has been a tendency to shortened bed rest, yet opinions are still divided on this point (9 12). The degree of severity of the infarction affects the prognosis (5 13 14) but from the literature no clear opinion emerges on the type of patients for whom a shorter period of hospitalization would suffice. There have been few reports based on a comparison of results of hospital stays of varying length.

In our hospital the period of bed rest and of total hospitalization has been considerably reduced recently. Our current therapeutic regimen is presented below and the mortality during one month of infarction is compared with the mortality of patients who previously had a longer hospital treatment.

MATERIAL AND METHODS

Series I

During the period Jan. 1 – April 30, 1969 the myocardial infarctions treated at our hospital were divided into two groups: severe (grade I), and mild (grade II) infarctions. A patient was considered to have severe infarction if one of the following findings was established.

1. SGOT above 150 units.
2. SHBD above 650 units.
3. Leucocyte count above 13,000 mm³ (excluding first value recorded at hospital).
4. Systolic blood pressure below 100 mm Hg, or in hypertensive patients less than two-thirds of their average value.
5. Axillary temperature 38°C or more in at least two measurements.
6. Chest pain lasting for more than [illegible]eek at hospital and not relieved by only occasional use of analgesics.

If none of the above criteria was demonstrable, the patient was classified as grade II. In case the patient died within two days of admission to hospital, without any of the above criteria being noted, the grade of severity was considered undetermined. All infarction patients were treated in the intensive-care unit at the early stage. The patients with severe infarction were kept in bed for 14 days; sitting up in bed was allowed during a period of 7 days, after which the patients were ambulant for 7 days before discharge. The hospital stay thus lasted 28 days. The patients with myocardial infarction classified as mild were kept in bed

Table I. *Characteristics of patients in two series*

	Men				Women			
	Series I		Series II		Series I		Series II	
	80		96		28		50	%
Age < 40	5/80	6	6/96	6	—	—	2/50	4
Age 40–70	69/80	74	73/96	76	18/28	64	29/50	58
Age > 70	16/80	20	17/96	18	10/28	36	19/50	38
Underweight	9/67	13	13/72	18	[illegible]/21	10	2/31	6
Overweight	18/67	27	19/72	26	10/21	48	14/31	45
Cholesterol above 300 mg/100 ml	13/67	19	17/84	20	5/25	20	7/42	17
Complicated infarctions	28/80	35	31/96	32	7/28	25	18/50	36
Recurrent infarctions	13/80	16	23/96	24	6/28	21	9/50	18
Gr I infarctions	65/80	81	51/96	53	17/28	61	30/50	60
Gr II infarctions	12/80	15	38/96	40	11/28	39	19/50	38
Mean age (y)	60.0		58.3		65.8		65.[illegible]	

for only 7 days, but subsequent treatment was the same as for the severe infarctions. They remained at hospital for [illegible]1 days.

Series II

The infarction patients under our care during May 1–Aug. 31 1969 were divided into two groups according to whether the infarctions were intra- or transmural; the two types were differentiated on the basis of electrocardiographic changes. The patients were treated in the intensive-care unit for three days and then moved to the ordinary ward. The group of intramural infarctions was proved to coincide closely with the earlier group of mild infarctions (grade II).

The patients with transmural infarctions were given complete bed rest for 7 days, after which they were allowed to sit up in bed. Ambulation started on the 14th day and they were sent home two days later when hospitalization had lasted 16 days. Those with intramural infarctions were allowed to sit up in bed from the fourth day at hospital, and they were ambulant from the tenth day. They were discharged, like the others, two days later following a 12-day hospital stay.

Both the earlier and the later series included also complicated infarctions. A patient was placed in the group of complicated infarctions if, during hospitalization, total atrioventricular or bundle branch block, heart failure, pulmonary oedema, or hypotension developed which required specific treatment. The complicated infarctions were treated in the intensive-care unit until they could be transferred to the ordinary ward and treated along the same lines as the patients with severe (transmural) infarction. Occasionally, however, ward treatment had to be continued longer owing to the development of cardiac insufficiency or conduction disturbances.

In an attempt to discover the effect of abbreviated bed rest and hospital stay on the immediate prognosis of these patients, we conducted a comparison between Series I and Series II. Apart from the duration of bed rest and of hospital stay, the patients were treated similarly during the two periods and all of them received anticoagulant therapy while at hospital. In each of the two series the mortality at 7 days and 30 days from the onset of myocardial infarction was determined.

RESULTS

Table I compares the patients in the two series on the basis of given characteristics. In defining over- and underweight we utilized the ideal weights recorded by the Metropolitan Life Insurance Company (11) and the severity of the infarctions was evaluated in accordance with our earlier practice. In the later series (Series II) the figure for severe infarctions, among men, was lower and that for mild infarctions higher compared with the earlier series (Series I). In other respects the male materials did not differ essentially. For women a higher number of infarctions was recorded during the later period and the number of complicated infarctions was greater than during the first third of the year.

Table II compares the mortality of patients in the two series after 7 days and 30 days observation. In all groups the mortality was higher in Series I in which bed rest as well as total hospital stay were longer. The differences were not, however, significant.

Table III analyses the mortality of the men by degree of severity since the male series differed from one another as regards severity of the infarctions. Significant differences were not found between the two series in this respect either, but mortality is understandably lower in the case of infarctions of milder type (grade II).

The earlier series included 35 complicated infarctions, the later 49. The prognosis of these

Table II. *Short-term mortality after myocardial infarction*

	Alive after 7 days		Alive after 30 days	
	Men	Women	Men	Women
Series I	62/80 (78 %)	21/28 (75 %)	53/80 (66 %)	16/28 (57 %)
Series II	79/96 (82 %)	38/50 (76 %)	72/96 (75 %)	30/50 (60 %)

patients will be seen from Table IV. It is clear that the prognosis in complicated cases is poorer than for other infarction patients. The majority of deaths occurred at the early stage. Average duration of hospital stay for the survivors with complicated infarction was 28.4 days (range: 11–48 days) in Series I and 23.1 days (range: 13–45 days) in Series II.

DISCUSSION

As a matter of course every victim of coronary attack must enter hospital as soon as possible. The chief reason for immediate hospitalization lies in frequent occurrence of cardiac arrhythmias, which, if untreated, may lead to sudden death even if the myocardial damage as such is slight. The first few days—indeed first few hours—after infarction are the most dangerous. If the patient survives this early stage, his subsequent prognosis is, relatively speaking, much better. While treatment at hospital is definitely indicated, the part played in recovery by bed rest as such and by its duration is not at all so clear. Levine and Lown (8) were the first to show that the prognosis was, in fact, much better when patients were allowed to sit up in an armchair during the first ten days following myocardial infarction. Later several investigators have stressed the importance of early ambulation, but there is disagreement as to the question how soon after the onset of infarction ambulation should start.

Brummer and his co-workers have advocated early ambulation of patients with myocardial infarction in a number of reports (1–3). They gradually shortened the bed rest of these patients from an average of 16.2 days (in 1952–54) to 10.3 days (in 1962–64). According to

Table III. *Mortality after myocardial infarction by grade of severity. Men only*

	Alive after 7 days		Alive after 30 days	
	Grade I	Grade II	Grade I	Grade II
Series I	49/65 (75 %)	12/12 (100 %)	41/65 (63 %)	12/12 (100 %)
Series II	41/51 (80 %)	37/38 (97 %)	36/51 (71 %)	36/38 (95 %)

Table IV. *Mortality after complicated infarction*

	Complicated infarctions	
	Alive after 7 days	Alive after 30 days
Series I	1/35 (60 %)	16/35 (46 %)
Series II	34/49 (69 %)	23/49 (47 %)

these authors this did not impair the prognosis, and the average duration of hospitalization dropped from 22.6 days to 18.9 days. Their studies included no single case of serious thrombo-embolic complication. Groden et al. (4) studied the prognosis on the basis of 105 female patients with myocardial infarction. These were divided into two comparable groups: in one group the patients were mobilized slowly and stayed at hospital for five weeks, in the other mobilization was more rapid (duration of modified bed rest 14 days) and hospital treatment lasted three weeks. There was no difference between the groups in the short-term or long-term prognosis, and the writers concluded that, while greater freedom and earlier ambulation did not result in positive advantage to the patient, these measures were not harmful. Lauper et al. (6) made a comparative study of two groups of myocardial infarction patients. They treated 196 patients with strict bed rest, usually during 4–6 weeks (55 patients had a shorter period in bed from one to three weeks) and 297 patients underwent a programme of armchair treatment usually begun after the first week of infarction. There was no difference between the two series in fatality rates, neither during the first week (2_ %) nor the subsequent weeks (11 % versus 9 %). Causes of death and clinical complications were similar in both series, and the authors concluded that modified armchair treatment started after the first week of infarction is a safe routine procedure that can be applied to the majority of patients with uncomplicated infarctions.

In the present study the patients in each of the two series were divided into two groups according to the assumed severity of the disease. In the earlier series this was done on the basis of certain general criteria fo infarction, in the later on the basis of electrocardiographic changes. The patients with mild infarction in the earlier series were kept in bed for 7 days, and the

total hospital stay lasted 21 days; those with severer forms of infarction were treated with strict bed rest for 14 days and stayed at hospital for 28 days. In the later series the management of patients with mild (intramural) infarction included bed rest for three days and hospital care for 12 days, the same figures for patients with severe (transmural) infarction were 7 days and 16 days respectively The results seem to indicate that the division into mild and severe cases is justified, and the treatment of the mild cases was so successful during one month's observation (mortality 0–5%) that the results can hardly be much improved upon. When the two series are compared, the results at one month are found to be equally good in both. Thus it is evident that the shortening of bed rest and of hospitalization has not been detrimental The way in which early ambulation affects the long-term prognosis and the extent to which it can promote the return of the patients to their work are matters for future study In any case the abbreviated hospital stay has its advantages. The need for hospital beds decreases, the separation of the patients from their usual surroundings is shortened, and the serious nature of the disease is not unduly emphasized. In this way neurotic manifestations due to the disease can be avoided to extent; at present the mere diagnosis of ocardial infarction is a matter of horror to many patients, and a long hospital stay tends to neurotize them further The results of our studies support early ambulation and suggest that the appropriate length of hospital stay may even be shorter than hitherto recommended in the literature

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ESTIMATION OF THE SEVERITY OF AORTIC INCOMPETENCE FROM PROLONGATION OF THE LEFT VENTRICULAR EJECTION TIME

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Abstract. The regurgitant fraction of the total left ventricular stroke index was estimated by a new method in 30 patients with pure aortic incompetence and compared with cineaortographic findings. The total stroke index was derived from the left ventricular ejection time, heart rate, and aortic diastolic pressure. The assumption was made that the interrelationships between the four variables in aortic incompetence are not different from those, found by others, in normal subjects at rest and during exercise. The forward stroke index was determined by Fick's method. The regurgitant fractions varied between 0 and 75% and showed a close correlation to the angiographic severity of aortic incompetence. The correlation exceeded that offered by prolongation of the ejection time alone, aortic diastolic pressure alone, or aortic pulse pressure. The method seems suitable for estimation of aortic incompetence in the absence of significant aortic valve obstruction and even in the case of failing left ventricle. It is simple to apply as all the variables are easily determined at routine cardiac catheterization without using any additional equipment.

The left ventricular ejection time of the normal heart is a complex function of heart rate, left ventricular end-diastolic volume, myocardial contractility and aortic impedance. In normal subjects at rest and during exercise a strong relationship exists between the ejection time and the heart rate, which is by far the most important single determinant of the ejection time (19–21). Studies in experimental animals, where only one of the determinants of the ejection time has been changed while keeping the others constant, have shown that, in addition to the heart rate, changes of both the preload and the afterload as well as of the contractility induce significant changes of the ejection time (3–18). Similar relationships have been detected in intact human subjects (19) and a formula has been developed relating the instantaneous ejection time to the heart rate, the stroke index, and the aortic diastolic pressure (8).

In aortic incompetence the left ventricular ejection time is prolonged relative to the heart rate (2, 10–16, 19). It is obvious that the prolongation is due to the greatly increased total stroke volume to maintain a normal forward stroke volume despite the diastolic regurgitant flow (3).

So far no simple means has been available to estimate the total left ventricular stroke volume or the regurgitant fraction in aortic incompetence. However if the relationships found in normal individuals between the ejection time, heart rate, stroke volume and aortic diastolic pressure also hold true in aortic incompetence, it should be possible to derive the total stroke volume from the actually observed ejection time, heart rate, and aortic diastolic pressure. The forward stroke volume is easily measured at cardiac catheterization. By these means the regurgitant fraction could also be calculated. In the present study we have employed the known relationships between the ejection time and its determinants to estimate the total stroke volume and the regurgitant fraction in aortic incompetence. The accuracy of the method in assessing the severity of aortic incompetence has been tested by comparing the regurgitant fractions to the cineaortographic findings.

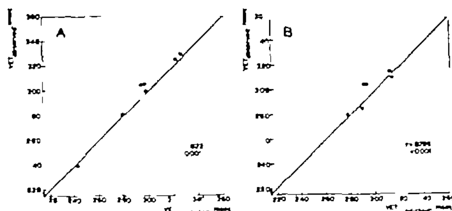


Fig. 1. Comparison of the observed and predicted left ventricular ejection times (LVET) in 26 subjects with normal heart or with mild to moderate mitral valve disease. *A* = prediction based on the formula of Jones and Foster. *B* = prediction based on the relationship to heart rate alone (21). Correlation is better when the formula is used.

MATERIAL AND METHODS

Thirty patients with pure aortic incompetence of varying degree were studied. Fourteen were females and 16 males in the age ranges 29–58 years and 21–64 years, respectively. The maximal peak systolic gradient across the aortic valve was 25 mm Hg. No coexistent lesion other than mild secondary mitral incompetence (8 cases) was present in the patients studied. In all but one the etiology was congenital or rheumatic and the severity of the aortic incompetence had probably been stable for several years before the study. One patient (no. 1) had bacterial endocarditis 6 months before he was investigated, by which time left ventricular failure had developed due to multiple perforations of the aortic valve cusps.

Hemodynamic data were obtained by right and left heart catheterization. The ascending aorta and the left ventricle were entered through brachial arteriotomy using Lehmann no. 7 catheter. The pressures were measured with strain gauge electromanometer (Sanborn 767 B) and recorded with Sanborn Poly-Beam photographic recorder using the paper speed of 50 mm/sec. The cardiac output was measured by Fick method, the reproducibility of which in our laboratory is 8 %. The body surface area was determined from the DuBois nomogram.

The following hemodynamic data were used: arteriovenous difference of oxygen content, heart rate, forward stroke index as determined by Fick method, pulse pressure and diastolic pressure of the ascending aorta, and the isovolumetric contraction time (ICT) and the ejection time of the left ventricle (LVET). The isovolumetric contraction time was measured from the onset of the systolic pressure rise in the left ventricle to the onset of the systolic pressure rise in the ascending aorta in at least five beats free from extrasystoles, and the mean value was expressed to the nearest 5 ms. The ejection time was measured from the onset of the systolic pressure rise to the incisura of the pressure pulse of the ascending aorta in five consecutive beats, and the mean value was expressed to the nearest 5 ms. In cases with atrial fibrillation ten complexes were analyzed. The incisura loses its sharpness in very severe aortic incompetence. In these cases a broadened notch or a clear-cut change of direction of the central aortic pressure pulse remains visible. In these cases the incisura was timed to the onset of the deformed incisural event to obviate undue exaggeration of the ejection time.

The formula of Jones and Foster (8) was used in the calculation of the total stroke index from the measured values of the ejection time, heart rate, and aortic diastolic pressure. The formula is:

LVET = 366.46 + 0.76 × stroke index (ml/m²) − 1.08 × heart rate (cycles/min) − 0.36 × aortic diastolic pressure (mm *Hg*)

The regurgitant fraction was calculated as a percentage ratio of the difference between the total and forward stroke indices to the total stroke index. Using the same formula an ejection time was also predicted to correspond to the measured values of the forward stroke index, heart rate and aortic diastolic pressure.

The formula is based on 207 simultaneous determinations of the ejection time, heart rate, stroke index, and aortic diastolic pressure in 20 healthy males at rest and at varying levels of submaximal exercise in the supine position. Jones and Foster found excellent correlation between the predicted and actually measured values of the ejection time at heart rates of 51–177 cycles/min. The error of estimate was 15.5 ms. To test this formula further we compared the predicted and actually measured values of the ejection time using for the prediction both the formula of Jones and Foster and the relationship between the ejection time and the heart rate alone (21), in another group of 26 subjects without heart disease or with mild to moderate mitral valve disease. The results of this analysis are shown in Fig. 1. The predicting value of the formula of Jones and Foster in this group was excellent and superior to that found when only the heart rate was used to predict the ejection time.

The cineaortograms were used for the evaluation of the calculated regurgitant fractions as an index of the severity of aortic incompetence. The contrast medium was injected just above the aortic valve and the cine films were obtained in single plane, with the patient in the right anterior oblique position, and at a speed of 80 frames/sec. The degree of aortic incompetence was estimated from the cineaortograms semiquantitatively in-

Table I. *The observed and calculated hemodynamic data of 30 patients with aortic incompetence*

Pat. no.	Heart rate (cycles/min)	Aortic diastolic pressure (mm Hg)	Pulse pressure (mm Hg)	Arterio-venous O_2 difference (vol. %)	Cardiac index (l/min/m² BSA)	Forward stroke index (ml/m² BSA)	Total stroke index (ml/m² BSA)	Regurgitant fraction (%)	LVET (ms)	ICT (ms)	ICT/LVET	Angiographic grade of aortic incompetence (1–4)
1	70	42	60	5.1	2.82	40	157	75	395	30	0.076	4
2	95	76	144	6.1	3.30	35	124	72	330	35	0.103	3
3	88	56	92	3.6	6.69	76	170	55	380	25	0.066	3
4	79	50	70	6.7	3.04	58	121	69	355	60	0.174	4
5	5	50	72	5.7	2.66	36	89	59	335	40	0.119	3
6	74	60	65	3.6	4.43	60	112	46	350	35	0.101	2
7	87	75	123	5.9	3.45	40	124	68	340	30	0.088	2
8	82	66	128	5.3	3.54	43	100	57	340	—	—	4
9	81	57	74	5.4	3.46	43	133	68	360	45	0.125	4
10	57	66	68	5.5	3.08	54	51	0	320	70	0.226	1
11	70	41	103	5.0	3.12	44	111	60	360	—	—	4
12	75	67	63	5.1	3.63	48	103	53	340	50	0.147	2
13	90	63	72	8.0	1.88	21	63	67	310	65	0.210	2
14	74	82	38	4.4	2.82	38	43	12	300	55	0.175	1
15	86	76	72	7.3	2.72	32	40	20	280	60	0.214	2
16	68	50	76	6.2	2.68	40	105	62	355	45	0.127	3
17	69	50	87	7.1	1.89	27	96	72	370	25	0.064	3
18	72	58	95	6.6	2.44	34	101	67	340	55	0.162	3
19	67	50	81	5.2	2.69	40	71	44	330	65	0.197	2
20	90	60	58	4.2	3.30	36	91	60	315	40	0.125	3
21	67	60	76	4.1	3.26	47	89	47	340	55	0.153	2
22	62	57	43	5.0	2.56	41	61	33	325	60	0.179	1
23	60	60	45	5.3	2.36	39	53	26	320	90	0.280	2
24	68	50	125	5.7	3.34	41	112	56	360	50	0.139	3
25	115	46	123	8.8	1.61	14	39	64	255	95	0.380	4
26	70	62	64	3.4	3.30	47	93	49	340	55	0.162	2
27	72	72	88	5.2	2.81	39	121	51	355	70	0.197	2
28	66	80	90	6.3	2.38	36	72	50	315	85	0.298	2
29	99	64	64	4.4	3.00	51	72	29	335	65	0.194	1
30	77	75	101	3.4	4.30	56	96	42	330	50	0.133	2
Range	37 115	41–82	38–144	3.4–8.8	1.61 6.69	14–76	39–170	0–75	—	—	0.066–0.380	—

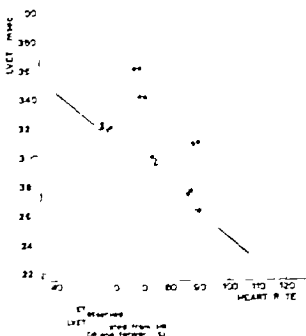


Fig. 2. Prolongation of LVET relative to heart rate in aortic incompetence. Normal range (mean ±1 S.D.) is from the data of Weissler et al. (20). Circles denote LVET predicted for each subject by using the formula of Jones and Foster.

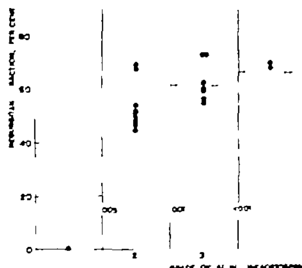


Fig. 3. The regurgitant fractions and their medians in the four angiographic grades of aortic incompetence. The *p* values are based on the Wilcoxon test between grade 1 and the other grades.

to 4 grades according to the shape and amount of the reflux and the proportion of the regurgitant valve plane of the total aortic valve plane, with some modification of the criteria of Hashimoto (7):

Grade 1. A small jet-shaped reflux, and regurgitant plane not more than one third of the total valve plane.

Grade 2. A fan-shaped reflux which does not completely opacify the left ventricle during several diastoles, and regurgitant plane not exceeding two thirds of the total valve plane.

Grade 3. A severe reflux without clearly delineated shape spreading at a wide angle and opacifying the left ventricle during a few diastoles, and regurgitant plane exceeding two thirds of the total valve plane.

Grade 4. A massive reflux opacifying the left ventricle by the end of a single diastole, and regurgitant plane exceeding two thirds of the total valve plane.

Differentiation between the first three grades was usually unequivocal and reproducible by two observers, but the differentiation of the borderline cases of grades 3 and 4 was less clear-cut.

RESULTS

The observed and calculated hemodynamic data are presented in Table I. If an arteriovenous oxygen difference greater than 5.5 vol.% at rest is used to indicate heart failure, this was present in 12 patients, later referred to as the heart failure group. The forward stroke index was below 35 ml/m² in five patients of this group. The ratio ICT/LVET, uncorrected for the heart rate, exceeded the upper limit of normal (9), 0.28, in two patients, was within the normal range in six patients, and below the normal lower limit, 0.12, in four patients. All 18 patients with an arteriovenous oxygen difference of less than 5.5 vol.% had normal forward stroke indices, and the ratio ICT/LVET was within normal limits in all but three patients whose ratio was below the normal range. Eight patients of 12 in the heart failure group and six of 18 without heart failure belonged to the angiographic grades 3 and 4.

Prolongation of the left ventricular ejection time relative to the heart rate is presented in Fig. 2. The predicted values of the ejection time based on the formula of Jones and Foster remained with few exceptions within the normal range relative to the heart rate. This occurred even in the presence of heart failure. The difference between the observed and the predicted ejection times varied from 0 to 89 ms.

The regurgitant fractions varied between 0 and 75%. The distribution of the regurgitant fractions in the four angiographic grades is presented in Fig. 3. Two-sample comparisons by

the Wilcoxon test showed that grades 2–4 were all significantly different from grade 1 Tested separately grade 2 differed significantly ($p < 0.01$) from grade 3 but no significant difference existed between grades 3 and 4 To evaluate further the results suggested by the Wilcoxon test, the ranked values of the regurgitant fractions in the four angiographic grades were treated by the Kruskal-Wallis test (14). This test gives a measure of association between the ranked variables and the angiographic grades, in principle like an intraclass coefficient of correlation. The association between the ranked regurgitant fractions and the angiographic grades was strong ($R^2 = 0.58$, $p < 0.001$)

The distribution of the relative prolongation of the ejection time in the four angiographic grades is shown in Fig. 4 Two-sample comparisons by the Wilcoxon test revealed significant differences only between grades 1 and 3 and between grades 1 and 4 while the other cross-comparisons showed insignificant differences. The ranked values of the relative prolongation of the ejection time showed a moderate association of borderline significance to the angiographic grade by the Kruskal-Wallis test ($R^2 = 0.31$ $p < 0.05$)

The distribution of the aortic diastolic pressure in the four angiographic grades is presented in Fig. 5 Two-sample comparisons by the Wilcoxon test showed a significant difference only

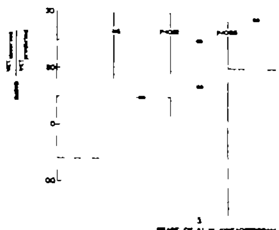


Fig. 4 The relative prolongation of LVET and the medians in the four angiographic grades of aortic incompetence. (The p values as in Fig 3.)



Fig 5 The aortic diastolic pressures and the medians in the four angiographic grades of aortic incompetence. (The p values as in Fig. 3.)

between grades 1 and 3 and between grades 2 and 3 ($p < 0.05$). The ranked values of the aortic diastolic pressure showed a moderate association of borderline significance to the angiographic grade by the Kruskal-Wallis test ($R^2 = 0.33$ $p < 0.05$).

The distribution of the aortic pulse pressure in the four angiographic grades is presented in Fig. 6. Two-sample comparisons by the Wilcoxon test showed that grades 2–4 differed significantly from grade 1 but the differences between grades 2 and 3 and between grades 3 and 4 were not significant. The ranked values of the aortic pulse pressure showed weak association of borderline significance to the angiographic grade by the Kruskal-Wallis test ($R^2 = 0.19$ $p < 0.05$)

It was concluded that the regurgitant fraction was definitely most powerfully associated to the angiographic grade of aortic incompetence and was superior to the prolongation of the ejection time alone, aortic diastolic pressure alone, or aortic pulse pressure. Furthermore, two-sample cross-comparisons demonstrated significant differences of the regurgitant fractions between all angiographic grades with the exception of grades 3 and 4 It is to be noted that the grading of the angiographic reflux was also difficult between these two grades.

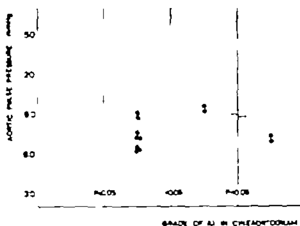


Fig. 6. The aortic pulse pressures and the medians in the four angiographic grades of aortic incompetence. (The p values as in Fig. 3.)

DISCUSSION

Assumptions in the formula

The formula of Jones and Foster used in the present study provides information of the interrelationships between the instantaneous left ventricular ejection time, heart rate, stroke index and aortic diastolic pressure in healthy males at rest and during submaximal supine exercise. Linear relationships are assumed to exist between the ejection time and its determinants. There is evidence from animal experiments that, within certain limits, each of the determinants, when altered separately, induces a linear change in the ejection tim (3, 18). Linear relationships are also present in intact man between the ejection time and the heart rate ranging from 45 to 150 cycles/min (1) and between the ejection time and the suddenly altered aortic pressure (13). However, the linearity of the relationship between the ejection time and the stroke volume (19) has been challenged by recent studies in patients with atrial fibrillation where the relationship is more closely described exponentially than linearly (6). Despite this, the error in assuming a linear relationship does not seem great. The formula ignores the ventricular contractile state which, however, will be indirectly included in the pooled data by its effects on the three determinants of the ejection time during exercise. Variation in the volume-pressure relationship of the ascending aorta and the small sex difference reported in the ejection time (20) are also ignored in the formula. Despite these limitations the formula seems to possess an excellent predicting value when applied to normal subjects (8) or to subjects without aortic valvular disease, as demonstrated in the methodical check up of the present study.

Application to stroke index estimation in aortic incompetence

The application of the formula of Jones and Foster to the estimation of the total stroke index in aortic incompetence must entail several assumptions. In general, the interrelations between the ejection time, heart rate, stroke index and aortic diastolic pressure in aortic incompetence should not greatly differ from normal. A few points deserve special consideration.

Firstly, variations in the contractility of the volume overloaded left ventricle should not influence the variables of the formula, especially the relationship between the ejection time and the stroke volume, in any way differently from resting and exercising normal subjects. Evidence is available indicating that the relationship between the ejection time and the stroke volume does not differ despite changes of contractility. The same regression line describes the relationship both in normal subjects and in subjects with heart failure (19), although a better correlation is found if an index of contractility such as the isovolumetric contraction time, is added to the regression analysis (1 0). When the end diastolic volume of the left ventricle has been increased while keeping the heart rate, aortic pressure and contractility unchanged, proportionate increases of the ejection time and the stroke volume occur in animal experiments. When the contractility alone is enhanced by positive inotropic agents, the ejection time will become shorter relative to the stroke volume (3, 18). However, there is no reason to assume that the contractile state in aortic incompetence would be enhanced. In experimental aortic incompetence the basic contractile state remains constant although the ejection fraction of the ventricle increases, solely due to reduction of impedance to ejection (17). From the evidence cited it may be concluded that in chronic aortic incompetence without heart failure a proportionate increase of the total stroke volume and the ejection time is to be expected. With de-

velopment of myocardial failure some prolongation of the ejection time relative to the stroke volume occurs, and the derived total stroke index will be greater than the actual. In the present study the presence of heart failure did not seem to cause overestimation of the regurgitant fraction relative to the angiographic grade (see Table I).

Secondly it must be assumed that the aortic diastolic pressure in aortic incompetence acts as similar index of aortic impedance as it does in normal subjects. The aortic impedance is a function of the aortic valve orifice, the aortic diastolic pressure, the volume-pressure relationship of the aorta, and the aortic run-off. No data are available of the volume-pressure characteristics of the aorta in aortic incompetence. Theoretically because the volume of the ascending aorta in chronic aortic incompetence is increased, it would seem that at least during the rapid ejection phase the resistance offered by the aortic wall would be less than normal. This would favor ventricular emtying and shorten the ejection time. The aortic run-off in aortic incompetence depends on the peripheral vascular resistance and the aortic reflux, and both factors are obviously important in determining the aortic diastolic pressure. The peripheral vascular resistance in aortic incompetence is usually reduced, but it increases above normal with development of left heart failure, with concomitant increase of the aortic diastolic pressure (12). The aortic valve orifice in aortic incompetence does not differ from the normal in its effect on the total aortic impedance, provided no cases with significant forward flow obstruction are present. Thus, several factors in aortic incompetence tend to lower the aortic impedance and thereby to shorten the ejection time in relation to stroke volume. These factors also lower the aortic diastolic pressure, which therefore reflects the aortic impedance probably with the same strength as in normal subjects.

Thirdly the ejection time in aortic incompetence, as determined from the aortic pressure pulse, should measure the same hemodynamic events as the ejection time in normal subjects. There is no available evidence that the inscription of the incisura would be timed differently from normal in aortic incompetence. With a very rapid aortic run-off in states with an extremely low peripheral resistance but without aortic reflux some delay of the incisura occurs. This is due to delay of the normal end-systolic cessation of the forward flow in the ascending aorta (11). Aortic incompetence will obviously act in the opposite direction and facilitate the reversal of flow. This occurs just before the aortic valve closure (15), and, therefore, the very last part of the ejection time, when measured from the pressure pulse, will actually include the start of regurgitation. Such an error in the definition cannot be of any practical value.

Comments on the results

Our method of estimating the total stroke volume and the regurgitant fraction in aortic incompetence necessitates the simultaneous determination of the cardiac output, heart rate, ejection time and aortic diastolic pressure. All this information is easily obtained in the routine cardiac catheterization. The regurgitant fractions in the present study varied between 0 to 75% of the total stroke volume. This range is fairly concordant with the range of 12 to 86% found by the indicator dilution technique in chronic aortic incompetence (5). The regurgitant fraction is obviously the most meaningful expression of the severity of aortic incompetence. Overlapping of the regurgitant fractions, notably only in the two most severe angiographic grades, may not be due to inaccuracy in the estimation of the regurgitant fraction, but rather to difficulties in angiographic interpretation in severe aortic incompetence. It is also fairly possible that the angiographic grades are not linear in terms of severity which also limits their use for comparison. Comparisons with quantitative methods are obviously desirable. It was not possible to consider the effect of a varying heart rate on the regurgitant fraction from the present data since the heart rate was directly involved in the calculations. When the heart rate is increased in aortic incompetence, the total stroke volume and the regurgitant volume will progressively decrease, and this introduces an inherent error into the angiographic estimation. However the regurgitant fraction remains constant irrespective of the heart rate (4), and thus no such error is involved in the estimation of the regurgitant fraction.

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SOLITARY PLASMOCYTOMA OF THE SPLEEN WITH MARKED POLYCLONAL INCREASE OF GAMMA G NORMALIZED AFTER SPLENECTOMY

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Abstract. A routine medical examination revealed markedly increased ESR in 40-year-old man. Further studies showed an enlarged spleen and marked polyclonal increase of serum globulins. Splenectomy was performed, revealing solitary plasmocytoma which weighed 1 kg. In the course f the first six weeks following splenectomy the serum proteins became practically normalized.

Primary extramedullary plasmocytoma is relatively rare. In Innes and Newall's (2) material of 188 cases of myelomatosis only four had primary extramedullary plasmocytoma, in no case located to the spleen. At autopsy however more than 20% of their patients with myelomatosis had gross extramedullary tumor masses within the abdomen or thoracic cavity in more than 10% of the cases also involving the spleen.

Dolin and Dewar (1) in 1956 made an extensive review of the literature on primary extramedullary plasmocytomas, 78% of which were located to the upper air passages and oral cavity Neither among their own nor among previously reported cases was there any instance of primary plasmocytoma in the spleen.

Villa (3) reported on a patient with plasmocytoma of the spleen as well as plasmocytic infiltration of the liver and bone marrow This patient had marked hypergammaglobulinemia with serum gamma globulins of about 6 g and total proteins of about 10 g per 100 ml. It is unclear whether the increase in gamma globulins was monoclonal or polyclonal (4). The markedly elevated gamma globulins remained unchanged for nine months following splenectomy but were normalized two years later Since that time the patient has remained in good health, with normal serum proteins (3 4). If one accepts Villa's patient as a case of plasmocytoma in the spleen, the patient most likely had widespread disease at the time of splenectomy The recovery a year after splenectomy might have been due to the removal of the prevailing splenic involvement.

We have been unable to find reports in the literature of primary plasmocytoma in the spleen without involvement of other organs.

The serum protein changes in solitary plasmocytoma are similar to those of generalized myelomatosis, but as a rule less pronounced, corresponding to the smaller number of plasma cells involved (6) In more than 150 personally examined cases of myelomatosis Waldenström saw no instance of polyclonal increase of immune globulins (6)

CASE REPORT

A man born in 1929 was admitted to the section of Hematology Rikshospitalet, on February .4, 1969 He had previously been in good health. During the last few months prior to admission, however he had felt that his usual energy was lacking. A medical examination in January 1969 by his family physician revealed sedimentation rate of 107 mm and total serum proteins of 9.5 g/100 ml with 4.3 g gamma globulins. The patient was then admitted to hospital for further studies.

Physical examination on admission revealed pea-sized lymph node on each side of the neck, otherwise no enlargement of lymph nodes. One had the impression of mass below the left costal margin, but physical examination was difficult because of well developed abdominal muscles. Physical examination was otherwise negative.

Laboratory findings Nitric acid ring test showed no proteinuria. Erythrocyte sedimentation rate was 100 mm, hemoglobin 13.3 g, erythrocytes 4.7 mill., leukocytes 6,600, platelets 770,000 per mm³



Fig. 1. The tumor tissue (shown on the right) is separated from the remaining splenic tissue by strands of connective tissue. In the tumor tissue plasma cells grow diffusely in a vascular stroma, completely effacing the normal splenic structures. Hematoxylin-eosin. ×[illegible]4.

Peripheral blood smear showed normal erythrocytes. Sixty per cent of the leukocytes were segmented neutrophil granulocytes, 30 [illegible] were lymphocytes. Bone marrow smear was slightly hypocellular. The erythropoiesis was normoblastic and amounted to 20% of the nucleated cells in the bone marrow. The granulocytopoiesis was normal in extent and distribution. Mature lymphocytes amounted to 1 % of the nucleated cells. There was no increase in plasma cells. The number of megakaryocytes appeared normal.

Bone marrow biopsy showed [illegible] cellular marrow with normal distribution of the different cell types and stages.

Biopsy of the enlarged lymph node on the neck showed chronic lymphadenitis, with only [illegible] few scattered plasma cells.

Antistreptolysin titer was 1,[illegible]40 (normal < 200). Antistaphylolysin titer 16 (normal [illegible] or less). Plasma fibrinogen 0.63 100 ml (normal 0.2–0.4). Total serum proteins were 10.1 g 100 ml (normal 6.5–8).

Serum electrophoresis showed [illegible] marked polyclonal increase in gamma globulins amounting to [illegible] g (normal 0.9–1.7). Immune electrophoresis showed a marked polyclonal increase in gamma G, quantitated to 5 g/100 ml. No increase in gamma A or in gamma M demonstrated.

Bromsulphalein test (5 mg kg body weight) showed 14% retention in 45 min. Serum glutamic pyruvic

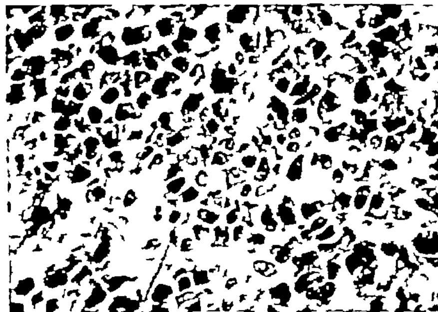


Fig. [illegible] This high-power micrograph illustrates how the plasma cells in the tumor are growing diffusely with only [illegible] few reticulum cells among them. The plasma cells are in general similar to normal plasma cells, although they vary more in size and configuration. Hematoxylin-eosin, ×645

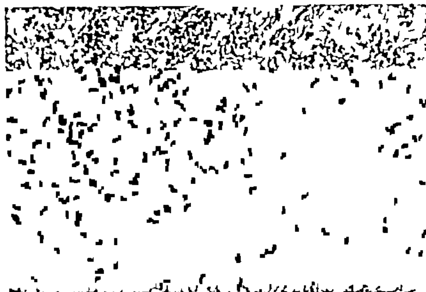


Fig. 3. This picture was taken from an area which macroscopically appeared white and translucent. It shows how plasma cells are lying interspersed in hyalin and acellular stroma resembling amyloid. Hematoxylin-eosin, × 4

transaminase (SGPT) was 5 U/L. Serum urea 36 mg, creatinine 0.8 mg/100 ml. Serum calcium 4.1 mEq/L, alkaline phosphatase 29 U/L. Roentgenogram of the chest normal. The tuberculin test was weakly positive. Intravenous pyelography showed normal kidneys and renal pelves. The splenic shadow however was seen to be enlarged with several calcifications. Celiacography verified the splenic enlargement. Roentgenograms of the skull, columna and pelvis were normal.

The nature of the splenic enlargement remained unknown, and splenectomy was done on March 6, 1969. The spleen weighed 1,030 g. In most places there was a small zone of normal splenic tissue beneath the capsule. The greater part of the spleen was replaced by a soft tumor which was separated from the surrounding splenic tissue by an irregular and thin capsule. The cut surface of the tumor was yellow or brown with scattered areas which had a rubbery consistency and a translucent, white appearance. Microscopically the peripheral splenic tissue showed no pathological changes. It was separated from the tumor tissue by strands of connective tissue (Fig. 1). The tumor itself was very cellular. The majority of these cells were plasma cells, but in some places many reticulum cells and groups of lymphocytes were also found. Some of the plasma cells were binucleated. Apart from that, they looked normal, and few mitoses could be found (Fig. 2). Hemorrhages and groups of hemosiderin-laden macrophages were present in several sections. Corresponding to the white translucent areas were found hyaline masses



Fig. 4. After staining with methyl violet many vessel walls showed metachromasia, as seen in the center of this picture. The vessels are surrounded by tumor tissue. Methyl violet, × 24.

Table I. *Serum proteins and some other laboratory values before and after splenectomy*

	Jan.	Feb.	Mar	Mar	Mar	Apr	May	Jun.	Aug.	Oct.
Date	14	25	6	19	4	22	6	18	6	7
ESR (mm)	107	100		55	55	12	5			
Total proteins (g/100 ml)	9.5	10.1		7.5	6.5	7.9	7.6	7.4	7.4	7.5
Gamma globulins (g/100 ml)	4.3	5.[illegible]	Splenectomy	2.4	1.9	2.3	[illegible].2	2.3	2.1	[illegible].0
Antistreptolysin titer		1280			320	40	200			240
Antistaphylolysin titer		16			4	2	2			1
Fibrinogen (g/100 ml)		0.63			0.63	0.41				0.43
Hemoglobin (g/100 ml)		13.3		12.7	12.1	15.1	15	15.6	16.7	16.0
Serum iron (μg/100 ml)		35			40	50				90
TIBC (μg/100 ml)		240			280	330				312

with groups of plasma cells (Fig. 3). Staining with Congo red showed only doubtful green birefringence when these areas were examined in polarized light. Similarly staining with Thioflavine gave an unspecific silvery blue fluorescence. On the other hand, both the hyaline masses and many of the vessels revealed typical metachromasia after staining with methyl violet (Fig. 4). These findings suggested that the hyaline substance could be amyloid, or paramyloid, deposits.

The patient developed transient paralytic ileus postoperatively but afterwards made an uneventful recovery.

In the course of the first six weeks following splenectomy the erythrocyte sedimentation rate, serum protein changes, antistreptolysin and antistaphylolysin titers all became practically normalized (Table I).

DISCUSSION

Roentgen celiacography as well as cross-sectioning of the removed splenic tumor showed a small brim of normal spleen at the upper end of the grapefruit-sized tumor. The tumor was sharply delineated from the normal splenic tissue and was quite solid. Microscopy showed diffuse infiltration of plasma cells, with some fibrous bands, scattered areas of paraamyloidosis, and complete extinction of all normal splenic tissue. There were no features suggesting Boeck's sarcoid, tuberculosis or other infection. A non-neoplastic plasma cell granuloma like those found in the conjunctiva, and occasionally in the upper air passages, is considered unlikely. The histological examination leaves little doubt about the diagnosis of plasmocytoma originating in the spleen.

The typical polyclonal increase of gamma G in our patient is highly unusual for a patient with plasmocytoma. Our patient also had a moderate increase in antistreptolysin as well as antistaphylolysin titer like many other patients with polyclonal increase of immune globulins (5). The pronounced increase of immune globulins, as well as the elevation of antistreptolysin and antistaphylolysin titres, became practically normalized within six weeks following splenectomy. There can therefore be little doubt about the spleen being the site of the abnormal gamma G production.

The polyclonal increase of immune globulins in our patient with solitary plasmocytoma does not fit with the well established concept of myelomatosis as a neoplastic proliferation of a single clone of plasma cells. Our patient resembles those rare cases of lymphoproliferative disorders with polyclonal elevation of immune globulins (5).

One might consider the possibility of two different types of neoplastic proliferation of plasma cells and lymphocytes: the usual type having all cells from the same clone and therefore with monoclonal increase in gamma globulins and a highly unusual type with proliferation of several different clones and therefore a polyclonal increase in gamma globulins.

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THE HAEMODYNAMIC EFFECTS OF ATRIAL PACING IN PATIENTS WITH ISCHAEMIC HEART DISEASE

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Abstract. The haemodynamic effects of atrial pacing in 49 patients with ischaemic heart disease are described. The chief effect is a progressive reduction in stroke volume. Systemic pressure is unchanged but filling pressures first fall and then rise at the faster pacing rates. Cardiac output also falls at faster rates. It is concluded that atrial pacing has no deleterious effects in the range of rates that is used clinically to control bradycardia without atrio-ventricular block, or to treat arrhythmias. It is preferable to ventricular pacing because it retains atrial transport.

The importance of the contribution of atrial systole in cardiac performance has been appreciated for a number of years (9). The technique of atrial pacing, however has only recently been used to increase the cardiac output when there is an inappropriate bradycardia without atrioventricular block (7 15) Pacing has also found application in the control of arrhythmias (6 11 24) and more recently the atrial method has been chosen (12). The technique is particularly useful in patients who have undergone recent cardiac surgery (7 15 26) or recent myocardial infarction (16, 22) when cardiac function is impaired.

In common with other workers (4 8, 14) we have used the atrial pacing method in the evaluation of patients with ischaemic heart disease (1 23). This has provided us with the opportunity of studying the haemodynamic effects of atrial pacing in a number of patients with heart disease. We report here only the changes that occur before the induction of angina. The further haemodynamic changes that accompany pain are outside the scope of this communication and will be reported in full later

MATERIAL AND METHODS

All of the patients attended the Out-patient Department of the National Heart Hospital because of anginal pain They all had electrocardiographic (ECG) abnormalities consistent with ischaemic heart disease. Those with evidence of other types of heart disease systemic hypertension and pulmonary disease were specifically excluded. All patients were in sinus rhythm. There were 49 in all, 43 male and six female, their ages ranging from 35 to 73 with a mean of 52.5 years. The severity of disease was graded from 1 to 4 grade 1 being the least severe with anginal pain only on extra exertion and grade 4 the most severe with pain at rest and minimal exertion. Grades 2 and 3 were intermediate. Table I shows the clinical features, chest X-ray ECG and coronary angiographic findings in all patients. The ECG was interpreted as follows. Q waves with symmetrical inversion of the T wave without change in the ST segment in any lead, or failure of progression of the R wave in the praecordial leads, was considered to indicate previous myocardial infarction. "Squaring off" of the ST segment or depression of that segment with T wave flattening or inversion were considered to be due to myocardial ischaemia.

Procedure

The study was carried out in the morning, the patient having had a normal breakfast and no premedication. They were asked not to take nitroglycerine on the morning of the study and other drugs, such as propranolol, were not taken for at least 72 hours before. The supine position was used. The ECG was monitored constantly for changes in rate, rhythm and form, particularly of the ST segment and T wave. The following catheters were introduced under local anaesthesia. A bipolar Zucker (United States Catheter Inc.) electrode catheter via an arm vein into the right atrium. A position was found where stable atrial pacing could be maintained requiring not more than 3 volts from an external battery powered pacemaker The most satisfactory position was with the electrode at the junction of the superior vena cava and the right atrium (Fig. I). This position had the advantage of producing rela-

Table I *Clinical features of 49 patients with ischaemic heart disease*

						ECG at rest		Findings on coronary angiography: Vessel						
Pat.	Age	Sex	Grade 1–4	Sign of LVD	CXR	Inf.	Isc.	R	L	AD	C	Collateral vessels present	Pain induced	V
1	49	♂	3	AS	N	A	AL						+	
2	58		1	—	N	—	AL						−	
3	70	♀	4	AS	E, Ca	I	AL						+	
4	54	♂	2	—	N	—	I						−	
5	65	♂	2	—	N	A	—						−	
6	50	♂	4	11R, 111	N	—	L	Oc	—	Oc	—	+	+	Yes
7	49	♂	3	AS	N	—	AL						+	
8	55	♂	1	—	N	—	I						−	
9	50	♂	4	—	N	—	IL	MS	—	—	—	−	+	Yes
10	71	♂	3	AS	N	A	L							
12	71	♂		—	E, V	AL	—						+	
13	46	♂	1	AS	N	—	AL						+	
14	45	♂	3	—	N	—	AL						+	
15	60	♂	2	11S	N	AL	—						+	
16	47	♂	2	111	N	—	AL	B	—	Oc	Oc	+	+	
17	57	♂	2	—	E	—	AL						+	
18	60	♂	3	—	N	AL	—						+	
19	47	♂	2	—	N	—	IL						+	
20	55	♂	2	—	N	—	I						−	
21	59	♂	2	—	N	I	—						−	
22	52	♂	2	11R, 111	N	—	A						+	
23	56	♂	1	AS	N	—	AL		N				−	
24	62	♂	2	—	N	—	AL						+	
25	56	♂	2	11R	Ca	—	AL	B	B	MS	B	−	+	
26	59	♂	3	11S	N	A	L						−	
27	41	♂	3	AS	N	AL	L	MS	B	Oc	—	+	+	Yes
28	58	♂	4	11S	N	—	AL		N				+	
30	58	♂	3	11R, 111	N	I	AL						+	
31	52		4	AS, 11R	N	A, I	A, I	Oc	—	MS	MA	+	+	Yes
32	54	♂	4	11R	E, VC K	A, I	AL	Oc	B	B	B		+	Yes
33	46		3	11R	N	A, I	—	MS	—	Oc	MS	+	+	
34	45		2	AS	N	—	I						+	
35	46	♂	1	—	E, VC	AL	—	MS	MS	MS	MS		+	
	49	♂	2	—	N			MS	MS	—	—	−	+	Yes
	39	♂	4	—	N	—	I L	—	—	MS	I		+	Yes
38	45	♂	3	—	N	—	I L	—	—	MS	MS	+	+	Yes
39	51	♂	3	11S	Ca	—	I	Oc	I	—	—	+	+	Yes
40	41	♂	2	—	N	A	AL						+	
41	56	♂	2	11S	N	—	I						+	
42	62		4	11R	N	I	AL	MS	—	Oc	B	+	+	Yes
43	57	♂	4	—	N	I	L	I	—	Oc	Oc	+	+	Yes
44	58	♂	3	—	E	—	L						+	
48	46	♂	2	—	N	—	AL						−	
49	55	♂	1		N	A, I							+	
50	59	♂	3		N	I		—	—	MS	MS	+	+	Yes
52	73	♂	2	—	N	—	I L	Oc.	—	Oc	—		+	
53	60	♂	3		Ca	—	I	I	—	—	MS	−		Yes
54	37	♂	2		N	—	A, I L						+	
55	45	♂	2	—	N	—	I						+	

Headings
LVD Left ventricular dysfunction
CXR Chest X-ray
Inf. Infarction
Isc. Ischaemia
R Right
Lt Left main
AD Anterior descending
C Circumflex
V Vineberg operation performed

Signs of left ventricular dysfunction
AS Atrial sound
11S Single second heart sound
11R Paradoxical splitting of the second heart sound
111 Third heart sound

Chest X-ray findings
N Within normal limits
E Cardiac enlargement
Ca Calcification of coronary artery
VC Pulmonary venous congestion
K Kerley' B lines

ECG findings
A Anterior
I Inferior
L Lateral
Right bundle branch block present

Coronary angiographic findings
Oc Occluded
MS Major stenosis
I Single minor st.
B Beaded appearance
Aortogram only



Fig. 1. Postero-anterior X-ray of the chest showing the position of the bipolar pacing catheter at the junction of the superior vena cava and right atrium.

tively normal P wave, and presumably also relatively normal mechanical events. With increasing pacing rate the PR interval lengthens. Fig. 2 illustrates these points. A number 8 twin-lumen USCI (United States Catheter Inc.) catheter was inserted into a second arm vein and under fluoroscopic control passed to the pulmonary artery so that the tip of the catheter was impacted in the pulmonary wedge position, or a fine nylon catheter flow guided to the pulmonary artery. The choice of catheter depended on the size of easily accessible arm veins. A teflon tube (OD 1.6 mm, ID 1.1 mm) was passed percutaneously into the brachial artery and the tip advanced to the aortic arch. Pressures were measured using Consolidated Electrodynamics strain gauge transducers (Type 4-326-L212) and recorded on a Sanborn 964 four channel direct writing recorder. Mean pressures were obtained by electrical integration. Mean systolic ejection pressure was obtained by integrating the area under the arterial pressure curve from the beginning of the upstroke to the dicrotic notch and dividing it by the duration of systole measured in millimetres. The zero level for pressure measurements was taken as the mid-chest. Cardiac output was determined by the dye-dilution technique, 5 mg of indocyanine green being injected into the pulmonary artery and arterial blood withdrawn with a Kipp and Zonen constant rate pump through a Gilford densitometer using a Honeywell recorder. The down-stroke of the curve was re-plotted on semi-logarithmic paper and extrapolated to zero to eliminate the effects of re-circulation. The area under the curve was measured by planimetry. The following haemodynamic variables were measured: heart rate, cardiac output, mean pulmonary arterial, mean aortic, mean pulmonary capillary venous and mean right atrial pressures. Tension time index was calculated as the product of mean systolic ejection pressure, systolic ejection time, and heart rate. Left ventricular work is the product of cardiac output and mean systemic pressure. Left ventricular stroke work is the product of stroke volume and mean systemic pressure. Total peripheral resistance was obtained by dividing mean systemic pressure by cardiac output and the systolic ejection rate by dividing stroke volume by systolic ejection time.

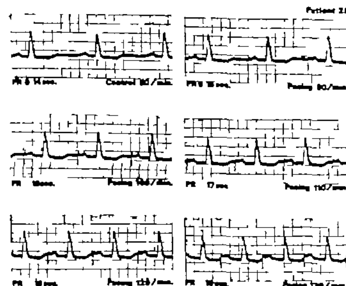


Fig. 2. The ECG showing prolongation of the P-R interval with increase of the pacing rate from 90 to 130 beats/min.

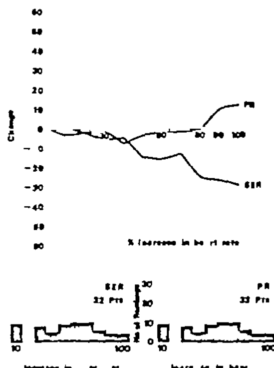


Fig 5 Percentage change in total peripheral resistance (PR) and mean systolic ejection rate (SER).

This is followed by a rise in left sided filling pressure. Data was not available for right atrial pressure at the higher heart rates. However this was showing a tendency to rise, with 70% increase in rate. The initial fall in both right atrial and pulmonary capillary venous pressures is consistent with the reduction in stroke volume according to the Starling mechanism (25). At faster heart rates, when the cardiac output is falling, filling pressures show a tendency to rise, the left more than the right. Although left atrial pressure is normally higher than right, the difference between the two here was perhaps greater than expected, especially at the higher rates. This may be a reflection of the decreased compliance of a diseased left ventricle compared with the right in these patients with ischaemic heart disease.

Mean systolic ejection rate has been used as an indicator of left ventricular function since it is an easily determined variable that bears some relationship to the more fundamental variable, the velocity of fibre shortening. It has been shown (10) that ventricular dimensions are reduced when the heart rate is increased by pacing. It might therefore be expected that the force and thus velocity of contraction would be reduced with increasing rate according to the Starling mechanism, and this would explain the observed reduction in systolic ejection rate. It has been pointed out that the velocity of shortening is in fact increased with increasing rate (17) since a greater degree of fibre shortening has to occur for a given stroke volume if the initial fibre length is reduced. It is thus difficult to ascribe changes in ejection rate to variations in left ventricular function.

The patients reported here are a mixed group with ischaemic heart disease ranging from the mildest form to those severe enough to require operative treatment. They were all, however, considered to be at risk from the point of view of the development of both bradycardia and ectopic ventricular activity at some time. These studies show that short term atrial pacing has no deleterious haemodynamic effects when used in a

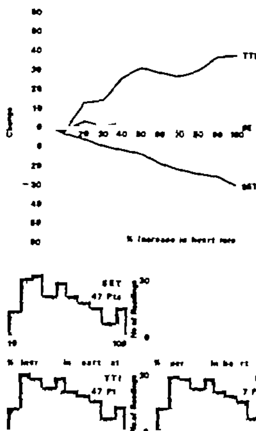


Fig 6. Percentage change in mean systolic ejection pressure (SE), systolic ejection time (SET), and tension-time index (TTI)

range normally required to suppress ventricular ectopic beats, which in our experience has been betw-en approximately 90 and 120 per minute. Since the PR interval increases with increasing pacing rate, the induction of "heart block might be anticipated. This phenomenon was not observed in this group of patients but we have seen it in a few patients studied since, at pacing rates of 150 per minute and above. The importance of atrial transport to patients with heart disease has b-en well demonstrated (3 13 .1), as have the advantages of atrial over ventric ular pacing (2, 5). These short term studies suggest that atrial pacing is the method of choice for the treatment of drug resistant bradycardia without atrio-ventricular block and also for ventricular ectopic activity that has not responded to the usual pharmacological agents.

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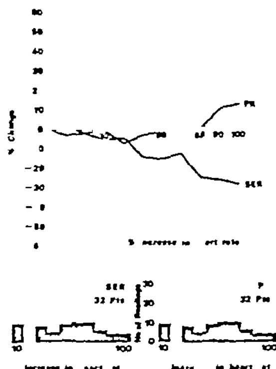


Fig. 5. Percentage change in total peripheral resistance (PR) and mean systolic ejection rate (SER).

This is followed by a rise in left sided filling pressure. Data was not available for right atrial pressure at the higher heart rates. However this was showing a tendency to rise with 70 increase in rate. The initial fall in both right atrial and pulmonary capillary venous pressures is consistent with the reduction in stroke volume according to the Starling mechanism (25). At faster heart rates, when the cardiac output is falling, filling pressures show a tendency to rise: the left more than the right. Although left atrial pressure is normally higher than right, the difference between the two here was perhaps greater than expected, especially at the higher rates. This may be a reflection of the decreased compliance of a diseased left ventricle compared with the right in these patients with ischaemic heart disease.

Mean systolic ejection rate has been used as an indicator of left ventricular function since it is an easily determined variable that bears some relationship to the more fundamental variable, the velocity of fibre shortening. It has been shown (10) that ventricular dimensions are reduced when the heart rate is increased by pacing. It might therefore be expected that the force and thus velocity of contraction would be reduced with increasing rate according to the Starling mechanism, and this would explain the observed reduction in systolic ejection rate. It has been pointed out that the velocity of shortening is in fact increased with increasing rate (17) since a greater degree of fibre shortening has to occur for a given stroke volume if the initial fibre length is reduced. It is thus difficult to ascribe changes in ejection rate to variations in left ventricular function.

The patients reported here are a mixed group with ischaemic heart disease ranging from the mildest form to those severe enough to require operative treatment. They were all, however considered to be at risk from the point of view of the development of both bradycardia and ectopic ventricular activity at some time. These studies show that short term atrial pacing has no deleterious haemodynamic effects when used in a

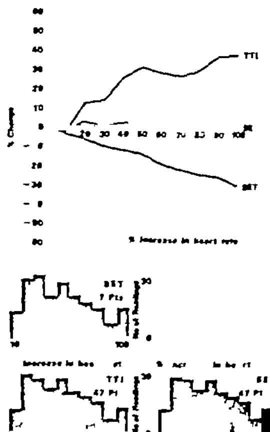


Fig. 6. Percentage change in mean systolic ejection pressure (SE), systolic ejection time (SET), and tension-time index (TTI).

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MORTALITY OF PATIENTS WITH ATRIAL FIBRILLATION BEFORE AND AFTER INTRODUCTION OF DC COUNTER SHOCK THERAPY

A Comparative Four year Study

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Abstract. In the series of 218 patients with atrial fibrillation treated by DC reversion 78 patients (35%) maintained sinus rhythm for three months or more. Twenty eight patients of this group have been followed up for four years, and the mortality rate was 32% in this group. The control series of 130 patients without DC reversion reached the same mortality level after only two years. This difference is highly significant. Problems concerning the choice of patients suitable for DC reversion is discussed.

It has long been known that 2.5–4% of patients with atrial fibrillation die suddenly and unexpectedly (2). Since electrical counter shock therapy was introduced and became extensively used, attention has been focused solely on factors associated with this form of therapy while the dangers of atrial fibrillation in a cardiac or other disease have been less studied. Reports on long-term results of DC reversion have recently begun to be available, and they provide information on the mortality rates of patients with atrial fibrillation. Radford and Evans (4) followed up 119 patients for two years, during which time two patients died. McCarthy et al. (3) reported on a series of 149 patients of whom only one died during the follow-up period of three years. Cramér (1) followed up an unselected series of patients reversed with quinidine therapy of a minimum duration of 8 months. Forty-four of her 132 patients with rheumatic heart disease (33%) and 28 of 105 patients without rheumatic heart disease (27%) died in the follow-up period. These widely differing mortality rates in different treatment of atrial fibrillation raise the question as to whether in a less selected series of patients treated with DC counter shock therapy the mortality really is so decisively low as found in a selected material. Moreover it is not yet known how the DC reversion affects the mortality rate and whether, if studied on an unselected series, it may be considered to prolong the life of cardiac patients with atrial fibrillation.

MATERIAL AND METHODS

The control series consisted of all the patients with diagnosis of atrial fibrillation treated at the Oulu University Department of Medicine from January 1, 1959 to December 31 1963. The interval from diagnosis of atrial fibrillation to the date of death was ascertained. With few exceptions these patients were on digitalis therapy and had not received anticoagulants or quinidine. No attempts at conversion by means of quinidine had been made. The distribution of the control series by age and sex is shown in Table I.

The series of the defibrillated patients started from April 1, 1964 when electrical counter shock therapy was introduced in our hospital, and continued until May 31, 1969. The methods and results concerning DC reversion have been published for both the early (6) and the late (5) phases. As a rule defibrillation was carried out unless contraindicated by an active cardiac process, prognosis which was poor in any case, digitalis intoxication, or electrolyte imbalance. A large left atrium of the heart, or generally large volume judged from the radiographs, did not preclude defibrillation, nor did possible latent heart failure. The vast majority of the patients, at the time of defibrillation, received both digitalis and anticoagulants, and 80% received quinidine. After defibrillation the patients were left on digitalis. Anticoagulants were discontinued immediately on defibrillation, apart from a few patients with persistent

Table I. *Age sex heart volume and etiological diagnosis of the total series*

			Sex		Heart volume[a]			Diagnosis				
	No of cases	Mean age (y)	♀ ( )	♂ (%)	N (%)	L ( )	G (%)	MI MS AS (%)	CHD ( )	TTHD ( )	HHD ( )	IAF ( )
Control series	130	58½	48	52				15	15	7	7	56
Total defibrillated series	218	56	52	48	10	62	28	[illegible]	21	9	10	31
No sinus rhythm, or sinus rhythm lasting less than 3 mo.	143	56½	51	49	7	65	28	[illegible]	22	10	11	29
Sinus rhythm lasting more than 3 mo	78	54½	56	44	15	56	29	30	19	13	11	21

Heart volume: N ♀ < 450 ml/sq m., N ♂ < 500 ml/sq m. L ♀ 450–700 ml/sq.m., L ♂ 500–750 ml/sq m., G ♀ > 700 ml/sq m. G ♂ > 750 ml/sq.m.

Diagnosis: MI mitral insufficiency MS = mitral stenosis, AS aortic stenosis, CHD = coronary heart disease TTHD treated thyroid heart disease, HHD = hypertensive heart disease IAF idiopathic atrial fibrillation.

atrial fibrillation who had earlier been affected with thromboembolism. After DC reversion the patients were given quinidine for three months or as long as the sinus rhythm lasted (5). For investigation of the present problem 218 defibrillated patients were followed up for three months. The distribution of the series according to whether the sinus rhythm had lasted three months, and according to age, sex and diagnosis, can be seen from Table I. The date of death of the defibrillated patients was traced and the interval from the moment of DC reversion was calculated.

The significance of the differences was studied by Student t-test.

RESULTS

The mortality rates of the following subgroups as a function of time from one up to four years were recorded: the control series, the total defibrillated series, the patients in whom the sinus rhythm either failed to return or lasted less than three months, and those in whom the sinus rhythm lasted more than three months. The mortality percentage of the total defibrillation series differs significantly ($p < 0.01$) from the control series only in the first and second years of follow-up. The mortality rates of the group in which the sinus rhythm either failed to return or lasted less than three months was not much different from that of the control series. But the mortality percentage of the group in which the sinus rhythm lasted for more than three months differed highly significantly ($p < 0.001$) from the control series up to the third year of follow-up, and during the same period significantly ($p < 0.01$) from the group in which the sinus rhythm either failed to return or lasted less than three months. Detailed figures are presented in Table II and Fig. 1

Table II. *Mortality rate during follow-up period*

	Less than 1 mo.	1 year	2 years	3 years	4 years
Control series	0/130 (0)	26/130 (20)	41/130 (32 %)	49/130 (38)	59/130 (45)
Total defibrillated series	6/218 (2.8)	20/218 (9) $p_1 < 0.01$	31/185 (17) $p_1 < 0.01$	43/134 (32) $p_1 > 0.05$	37/99 (37 %) $p > 0.05$
No sinus rhythm, or sinus rhythm lasting less than 3 mo.	6/143 (4)	20/143 (14 %) $p_1 > 0.05$	29/124 (23) $p_1 > 0.05$	37/95 (39 %) $p > 0.05$	28/71 (39 %) $p_1 > 0.05$
Sinus rhythm lasting more than 3 mo	0/75 (0)	0/75 (0 %) $p_2 < 0.001$ $p_1 < 0.001$	2/61 (3) $p_2 < 0.001$ $p_1 < 0.001$	6/39 (15) $p_2 < 0.01$ $p < 0.001$	9/28 (32 %) $p_2 > 0.05$ $p_1 > 0.05$

p_1 = significance compared with the control series.
p_2 = significance compared with the series without sinus rhythm or with sinus rhythm lasting less than 3 months.

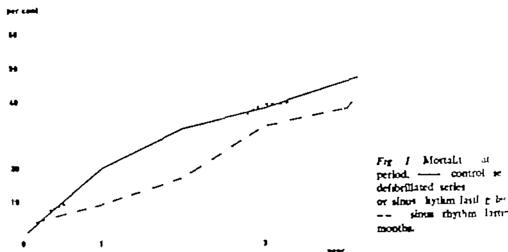


Fig. 1 Mortali at period. —— control se defibrillated series or sinus hythm lasti g be -- sinus rhythm lastin months.

The period during which the groups studied reached a given mortality percentage can be seen from Fig. 1 Considering that the statistically significant difference disappears somewhere between the third and fourth years of follow-up the group in which sinus rhythm lasted more than three months reached the mortality level of the control series about two years later Compared with the total defibrillated series the group in which the sinus rhythm lasted over three months reached the same mortality rate about one year later and compared with the group in which the sinus rhythm failed to return or lasted less than three months the difference was about 18–24 months.

Heart volume determined from the radiograph of the chest was distributed into normal (for women <450 ml/sq.m., and men <500 ml/sq.m.), large (for women 450–700 ml/sq.m., and men 500–750 ml/sq.m.), and giant (for women >700 ml/sq.m., and men >750 ml/sq.m.) The heart volume, classified on this basis, is evenly divided without statistically significant differences among the various subgroups of the material (Table I).

On the basis of the diagnosis the series was divided into the following groups: valvular heart disease, coronary heart disease, treated thyrotoxic heart disease, hypertension, and idiopathic atrial fibrillation, or idiopathic heart disease. The mortality rates of these groups were followed up in the total series, the defibrillated patients separately No statistically significant differences were recorded (Figs. 2 and 3) A pr a version of atrial fibrillation i to inu h successful in 87.5%

DISCUSSION

Owing to the few absolute contraindication plied, the present series comprised the ma ty of the patients with atrial fibrillation admitted to our hospital. Even when the contraindica

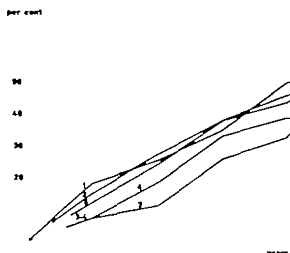


Fig. 2. Mortality rate of the whole material in different diagnostic groups 1 – CHD coronary heart disease. 2 – MI, mitral insufficiency – MS, mitral stenosis; – AS, aortic stenosis. 3 – TTHD, treated thyroid heart disease. 4 – HHD, hypertensive heart disease. 5 – IAF idiopathic atrial fibrillation.

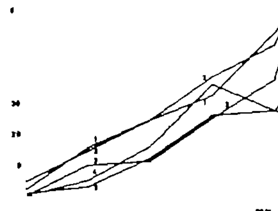


Fig 3 Mortality rate of the defibrillated series in different diagnostic groups. 1 – CHD coronary heart disease 2 – MI, mitral insufficiency – MS, mitral stenosis, – AS, aortic stenosis. 3 – TTHD treated thyroid heart disease. 4 – HHD hypertensive heart disease 5 – IAF idiopathic atrial fibrillation.

tions are few the series is partially selected, as can be seen from the remarkable difference in mortality rates between the defibrillated series and the control patients in the first two years. The selection usually tends to increase with the number of patients treated and when the methods of work have become established. The percentage of successful primary conversions reflects the selection. from the early percentage in our series, 84 (5 6) the figure has now climbed to 87.5%. The selection can also be assessed by comparisons. The mortality rate at 18 months in the present control series, and among those in whom defibrillation failed or sinus rhythm lasted less than three months, was roughly the same as in Cramér's unselected series (1) It seems that our DC reversion material represents a series as unselected as is possible when the DC reversion method is used.

The re-conversion of achieved sinus rhythm into atrial fibrillation seems generally to take place within about a month of the electrical counter shock (7). In view of the present problem a record had been kept of all patients as to whether the sinus rhythm still persisted 3 months after the reversion, for which reason this was chosen as the borderline date. We know from an earlier review that the next considerable relapse into atrial fibrillation takes place after 6 to 12 months, during which time the percentage of patients retaining the sinus rhythm falls from 34 to 13 4 (7).

The role of atrial fibrillation as a contributory factor towards mortality regardless of the etiological diagnosis has received little attention. Cramér's study on quinidine conversion was the first to quote a mortality rate of approximately 30 % after 8 months of follow-up. The present series confirmed this result and showed that the mortality rate of those in whom DC reversion failed to produce sinus rhythm approached 14% in one year The patients in whom DC reversion restored the sinus rhythm and who maintained it for a minimum of three months reached the 15 mortality rate two years later than did those in whom the DC reversion failed to produce the sinus rhythm.

The factors governing the formation of this group with a different prognosis or its characteristics are the keystone in studies of the role of DC conversion. The advantageousness of the sinus rhythm, both hemodynamically and in view of a possible thromboembolism, is the first to enter one's mind However in our series the number of patients who maintained the sinus rhythm began to fall considerably after 6–12 months, and at the end of a year their percentage was 14 Fatalities among those who acquired sinus rhythm after a DC reversion and maintained it for three months did not really start until two years after the reversion. The majority of these patients apparently have atrial fibrillation for over 12 months before the mortality rate steps up. It seems improbable that the sinus rhythm could account for the fact that the rise in mortality is delayed.

A second possibility is the selection. Its role begins with the choice of patients suitable for DC reversion. This is also suggested by our earlier review according to which primary failures and those occurring after four days were frequent in coronary disease and in myodegeneration. Among the groups in which the primary DC reversion failed, these two with potentially malignant diseases stand out more clearly than the others (7). Absence of selection is indicated by the even distribution of the different diagnostic groups and the heart volumes determined

from radiography into the various subgroups of DC reversion. The equality of the diagnostic groups was apparently due to the fact that the subgroups ultimately became too small. On the other hand, the cardiac volume may be a poor indication of the maintenance or non-maintenance of sinus rhythm or atrial fibrillation, and its coverage may be too extensive for the assessment of mortality. The selection which enters into the picture from the very beginning must apparently be borne in mind all the time during which the DC conversion and its results are followed up.

The role of DC conversion in the light of mortality behavior receives added emphasis. The sinus rhythm obtained by conversion is not enough to account for the better prognosis of these patients. The electrical counter shock therapy apparently selects the patients with good prognosis, characterized by a good defibrillation and maintenance of the sinus rhythm for a minimum of three months.

Bearing in mind that the sinus rhythm almost always disappears later and that electrical counter shock therapy is an arduous method of treatment, it may be questioned whether this therapy really is necessary and whether there might not be other criteria, easier to apply for choosing the patients with a good prognosis. A prospective comparative study might throw light on the role of DC reversion in the prognosis of patients with atrial fibrillation.

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PLATELET PHOSPHOLIPIDS AND THEIR FUNCTION IN PATIENTS WITH ISHEMIC HEART DISEASE

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Abstract. Platelet factor-3 activity and availability platelet phospholipids and their fatty acid and aldehyde composition have been examined in males with non-acute ischemic heart disease and in control group. Increased platelet factor 3 activity was present in platelet-rich but not in platelet-poor plasma from the patients. N difference in the increase of activity after exposure of PRP to ADP kaolin or freezing and thawing three times was observed compared with the controls. Estimated per 10^9 platelets, increased amounts of ethanolamine glycerides, serine phosphoglycerides, choline phosphoglycerides, sphingomyeline and total lipid phosphorus were found in the patient group. The phospholipid protein ratio was also increased. Only small changes were observed in the fatty acid pattern of the various phospholipids. A slight decrease in palmitic acid and slight increase in oleic acid were found in most fractions.

Changes in serum lipids and platelet function have been reported in the pathogenesis of ischemic heart disease (I.H.D.) (1 5 8, 10). However no clear correlation exists between any of the single parameters and the individual risk of developing coronary disease, although in larger groups of patients the cholesterol level in serum shows a positive correlation (8).

The tendency to thrombus formation in coronary arteries plays fundamental role in the development of I.H.D. Firstly thrombosis often represents the final occluding event in therosclerotic arteries. Secondly there is good evidence that mural thrombi may be transformed to atherosclerotic lesions (9 14 19). When an arterial thrombus is formed, adhesion of platelets to the vessel wall and to each other possibly initiates the sequence of events. These initial steps are followed by platelet reactions producing exposure on the platelet surf ce and release of coagulation-active phospholipids (platelet fa t 3). These lipids, which are located mainly platelet granules and membranes, participate in the coagulation reactions, eventually leading to the formation of fibrin which consolidates the thrombus (18).

In the present study we have investigated the vailability and clotting activity of platelet factor 3 and also compared the biochemical nature of the platelet phospholipids in normals and in patients with I.H.D.

MATERIAL

Venous blood was collected after 1 14 hours fasting. Medical and dietary history physical and hematologic examinations, including estimation of the main plasma lipid fractions, were carried out. The control group comprised 20 healthy male subjects between 30 and 60 years of age. The patient group comprised 15 male subjects between 47 and 69 years f age. Five had experienced myocardial infarction more than one year before they were tested. The diagnosis had been confirmed in all cases by typical electrocardiographic changes, elevation of transaminases, leucocytosis and increased sedimentation rate. The other ten had angina pectoris and showed ischemic changes in the electrocardiogram without or with exercise and prompt relief of pain by nitroglycerin. None of the subjects used other drugs and none were on dietary regimen.

METHODS

All the methods used have recently been described in detail (17). The outline of the procedures follows.

Preparation f platelets for lipid analyses

Thirty-six ml of blood was collected in 4 ml of 0.077 M EDTA solution, pH 6.4 chilled at 0°C and processed immediately. By differential centrifugation plate-

Table I. *Age, duration of symptoms and serum lipid levels in normals and in patients with I.H.D.*

	Normal (n 10)	I.H.D (n 15)
Age	49 (35–63)	59 (42–69)
Duration of symptoms (y.)		5 (1–10)
Total cholesterol (mg/100 ml)	287±44	344±33
Triglycerides (mg/100 ml)	98±49	146±59
Phospholipids (mg/100 ml)	208±28	257±32

Significance, $p < 0.01$

let concentrate was obtained which was washed twice. The platelets were counted in a celloscope before the extraction of lipids was carried out. Alpha-tocopherol was added to all samples to avoid autooxidation.

Estimation of PF 3 activity

Nine ml of blood was collected in one ml of a 0.106 M citrate solution. By centrifugation at 350 g for 15 min and at 2,200 g for 30 min platelet-rich (PRP) and platelet-poor plasma (PPP) were prepared. All samples were kept on melting ice until they were tested. Samples of PRP containing 300 10^3 platelets per µl and PPP containing 15 10^3 platelets per µl were used. The PF-3 activity was tested in a Stypven system using 0.4 ml plasma for each test. The availability of PF-3 was tested in PRP exposed to ADP and kaolin or frozen thawed three times.

Phospholipid fractionation

The phospholipid distribution was determined in triplicate by thin layer chromatography (TLC) in nitrogen atmosphere. Various staining procedures, standard phospholipids and two-dimensional TLC were used for identification. The amount of lipid phosphorus was determined by Marinetti's modification (13) of Bartlett's method (2) and the average recovery on TLC was 93.8%.

Table II. *Stypven time in platelet-rich and platelet-poor plasma from normals and in patients with I.H.D.*

No. of platelets/mm³	Test reagent	Stypven time (sec) Normal (20)	I.H.D (n 10)
300,000	Buffered saline	43.1±4.5 (34.7–56.6)	36.5±2.5 (32.3–39.5)
300,000	Kaolin (1 mg/ml)	29.1±4.0 (21.8–36.6)	27.2±3.4 (24.4–34.9)
300,000	ADP (1 10^{-4} M)	34.4±3.9 (25.5–43.9)	33.7±2.8 (30.3–39.8)
300,000	Frozen and thawed three times	14.9±1.9 (12.7 19.0)	16.2±3.0 (13.9–21.4)
15,000	Buffered saline	59.5±9.3 (44.4–84.1)	58.7±6.0 (52.3–72.6)

Significance, $P < 0.01$

Estimation of the phospholipid fatty acids and aldehydes

Methyl esters and dimethyl acetals were prepared from the phospholipid spots from the TLC procedure by boron trifluoride methanol. Gas liquid chromatography (GLC) was performed on an F & M Scientific Instrument Model 402 equipped with paired glass columns, 6 ft × 5 mm i.d. and packed with 12% DEGS on 80–100 mesh Chromosorb W. Programmed runs from 165 to 195 were used. Peak areas were calculated with planimeter and NIH fatty acid methyl ester standard mixtures were used to control the quantitation and as references.

Protein analyses

The protein estimation was carried out on aliquots of washed platelet concentrate using Lowry et al.'s method (12) as modified by Miller (15).

RESULTS

All subjects were in good general condition at the time they were tested. No detailed dietary examination was performed, but all were on an ordinary Norwegian diet without any restrictions or supplements.

The average age in the I.H.D group was higher than in the control group. The serum levels of total cholesterol and phospholipids were significantly higher in the patients, as shown in Table I. Lipoprotein electrophoresis showed a normal pattern of the serum lipoproteins in six of the patients with I.H.D., five had increased β-lipoproteins, and four had increased pre-β-lipoproteins. No clear correlation could be established between the lipoprotein pattern and the platelet phospholipids, but the subgroups are too small to exclude such a connection.

Platelet factor 3 activity

The coagulant activity of platelets measured in a Stypven system showed that the I.H.D group had an increased activity of platelet-rich plasma with a standardized platelet number (Table II). After exposure of the platelets to kaolin or ADP no significant differences were observed. The total activity measured after the PRP was frozen and thawed three times and the "zero" activity measured in PPP were not different in the two groups.

Table III. *Phospholipid composition of platelets from normals and patients with I.H.D*

Compound	Normal (n 20) (µg lipid–P/ 10^9 platelets)	(%)	I.H.D (13) (µg lipid–P/ 10^9 platelets)	%
P.E.	3.00±0.28	31 6	3.63±0.67	30.9
P.S.	0 63±0.11	6.7	0.94±0.23	8.2
P.I.	0.32±0 09	3.4	0.39±0.11	3 4
P.C.	4.22±0.32	44.7	5 17±0.86	43 7
SPH.	1.28±0.17	13.5	1.59±0.40*	13.5
Tot. rec. lipid–P	9 45±0 64		11.72±1.33	

Significance, $P < 0.01$.
Significance, $P < 0.05$.
Moles/100 moles of total recovered phospholipids.

Table IV *The phospholipid. protein ratio i platelets from normals and patients with I.H.D*

	Normal (n 10)	I.H.D. (n 12)
Phospholipid (mg) / Protein (mg)	0.14±0.02	0.17±0 04

Significance, $P < 0.01$

Table V *Fatty acid distribution (g/100 g of fatty acids and aldehydes) of et   l   h phosphoglycerides and serine-phosphoglycerides in platelets*

Components	Ethanolamine phosphoglycerides Normal (n 10)	Ethanolamine phosphoglycerides I.H.D (n 14)	Serine-phospho-glycerides Normal (10)	Serine-phospho-glycerides I.H.D (14)
16 0, 16 0 DMA	15.0±3.6	13.6±3.4	11.8±6.3	7.3±2.8
16 1	0.7±2.4	1.2±1.0	1 1±0 2	0.6±0.1
17 0	0 6±0.2	0.4±0.1	0.4±0.1	0.4±0.1
18 0, 18 0 DMA	27.3±6.8	31.0±7.6	38.9±8.5	44.1±8.4
18:1 18 1 DMA	11.9±2.4	13.8±4.6	22.8±4.3	25.7±4..
18 2	3.2±1.2	3.1±0.9	2.8±0.7	2.1±0.9
20:0	0.5±0.1	1.3±1.0	1.5±0.4	1 4±0.5
20 1 18 3	0.8±0.3	0.7±0.2	0.3±0.1	1.2±0.4
20 3 22:0	1.3±0.2	1.2±0.5	1.8±0.3	2.0±1.1
20 4 22 1	23.8±8.2	23.7±8.5	11.3±5.0	13.0±7.3
20:5 22:2	1.5±0.3	1.2±0.6	0.9±0.3	0.4±0.1
22 4	2.5±0.9	1.5±0.3	Trace	Trace
22:ron	2.8±0.5	2.2±1.8	Trace	Trace
22 5	3.9±1 1	2.1±0.8		
22 6	4.9±1.3	3.1±2.7		
26 1	0 9±0.2	1.0±0.2		

Significance, $P < 0.01$.
Significance, $P < 0.05$.

Phospholipid composition of the platelets

Tables III and IV show the mean values and the standard deviation of the total lipid phosphorus, the phospholipid distribution and the phospholipid protein ratio of platelets from normals and from patients with I.H.D

A significantly higher content of total phospholipids was found in platelets from patients with I.H.D., and the phospholipid protein ratio was also significantly increased. The high total phospholipid content reflected increased amounts of the ethanolamine-phosphoglycerides (PE) serine-phosphoglycerides (PS), choline-phosphoglycerides (PC) and sphingomyeline (Sph). The per centage distribution of the various phosphol pi..s was not significantly changed.

Fatty acid composition of platelet phospholipic

The fatty acids and aldehydes of the four main phospholipid fractions were quantitated, and the results are given in Tables V and VI

The fatty acid pattern was much the same in the two groups. The level of oleic acid (18 1) usually was increased in the platelet phospholipid from the patient group whereas palmitic a id (16 1) was decreased. The ratio of saturated acids plus aldehydes to unsaturated fatty acids was not significantly changed in any of the pa tients with I.H.D (Table VII)

DISCUSSION

The present study has shown that patients with ischemic heart disease without recent myocardial infarction have a higher coagulant activity in platelet-rich plasma than the controls. As there is no difference between the activity in platelet poor plasma of the two groups, this activity must be attributed to the platelets. This is in accordance with earlier observations (3 20) Increased amounts of phospholipids per platelet, both absolute and when related to the protein content, were found in patients with ischemic heart disease. As these phospholipids, particularly phosphatidyl ethanolamine and phosphatidyl serine, may substitute platelets in the in vitro clotting system used, we suggest a relation between the increased phospholipid content and the increased clotting activity in platelet-rich plasma. The platelet factor 3 activity is more easily brought on by the mechanical strain induced during pre paration of PRP

Table VI. *Fatty acid distribution (g/100 g of fatty acids and aldehydes) of choline-phosphoglycerides and sphingomyeline in human platelets*

Components	Choline-phosphoglycerides Normal (10)	Choline-phosphoglycerides I.H.D. (13)	Sphingomyeline Normal (10)	Sphingomyeline I.H.D. (14)
16:0	33.9 ± 7.9	32.8 ± 3.[illegible]	18.8 ± 10.[illegible]	20.9 ± 5.7
16:1	1.5 ± 0.5	1.8 ± 0.9	1.4 ± 0.1	1.0 ± 0.2
17:0	0.4 ± 0.1	0.3 ± 0.1	[illegible]0 ± 0.6	0.[illegible] ± 0.1
18:0	16.3 ± 1.6	15.6 ± 1.9	13.4 ± 7.8	7.1 ± [illegible]8[a]
18:1	23.[illegible] ± 4.8	[illegible]5.7 ± [illegible]9	8.7 ± 4.5	6.4 ± 3.4
18:2	6.5 ± [illegible]4	7.5 ± 1.3	0.4 ± 0.1	0.8 ± 0.[illegible]
20:0	1.3 ± 0.[illegible]	1.4 ± 0.[illegible]	4.5 ± [illegible]4	4.9 ± [illegible]9
20:1, 18:3	[illegible]1 ± 0.4	3.0 ± 0.8	Trace	1.1 ± 0.[illegible]
20:3, 22:0	1.6 ± 0.3	1.8 ± 0.5	15.6 ± 5.3[a]	19.5 ± 4.1
20:4, 22:1	10.3 ± 5.5	8.5 ± 3.1	13.9 ± 4.9[b]	10.7 ± 4.6
20:[illegible] 22:[illegible]	0.9 ± 0.[illegible]	0.4 ± 0.1		
22:4	0.9 ± 0.[illegible]	Trace		
24:0			8.3 ± 4.4	11.2 ± 4.5
24:1			14.1 ± 3.8	15.9 ± 4.7
22:[illegible]	0.3 ± 0.1	Trace		
22:5	1.7 ± 0.3	Trace		

[a] Significance, $P < 0.01$.
Appears to be largely 22:0.
[b] Appears to be largely 22:1.

When platelets are exposed to aggregating substances like ADP or kaolin, the platelet phospholipids are made partially available for the plasma coagulation system. A similar reaction to these substances was found in platelets from normals and patients with ischemic heart disease. When platelets are frozen and thawed three times, probably all phospholipids are made available. Although increased amounts of phospholipids were present in platelets from the patients, no increased activity could be estimated in the Stypven system after the freezing and thawing procedure compared with normals. Most likely this reflects the insensitivity of the method for discrimination between varying values at *high* concentrations of platelet factor 3 (17).

To explore whether chemical differences of the single platelet phospholipids might be responsible for the difference in clotting activity of platelets from normals and the patients, their fatty acid and aldehyde pattern was examined. A tendency to increased amounts of stearic acid and oleic acid and a decreased amount of the highly unsaturated long chain fatty acids was found in most fractions. However, great individual variations were present both in normals and in patients, and the difference between the two groups thus becomes non-significant. We could not find a similar change in the platelet cephalins (PE–PS) as reported for plasma cephalins (3) in patients with I.H.D.

Earlier studies have shown that patients with ischemic heart disease have larger platelets than normals (6). These large platelets might belong to the young, metabolic active platelet population (11), indicating an increased platelet turnover in patients with I.H.D. (16). However, platelet lifetime studies (7) and studies on ADP platelet reaction in patients with ischemic heart disease (4, 5, 6, 21) have given conflicting results and have been difficult to evaluate, probably because of methodological differences. The present study supports the view that platelets from patients with ischemic heart disease are structurally and metabolically different from platelets in normals in such a way that they favour the tendency to thrombosis.

Table VII. *Percentage composition of fatty chains of platelet phospholipids in normals and patients with I.H.D.*

Compound	Saturated fatty acids + aldehydes / unsaturated fatty acid: Normal	I.H.D.
P.E.	0.89	0.91
P.S.	1.18	1.13
P.C.	1.05	1.00
SPH	1.54	1.6

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GASTRO-INTESTINAL DISTURBANCES IN PATIENTS WITH SEVERE RHEUMATOID ARTHRITIS

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Abstract. Twenty-two patients with classical rheumatoid arthritis (RA) and two with active rheumatoid spondylitis have been investigated for malabsorption. The liver and pancreatic functions were also studied. Ten patients had flat lactose tolerance curves. In six of these, intestinal biopsy revealed selective lactase deficiency. Intestinal amyloidosis was observed in four patients, while a fifth showed renal amyloidosis. Seven patients had pathological d-xylose tolerance tests and nine had pathological vitamin A tolerance curves. Five patients exhibited folic acid deficiency and twenty low serum iron levels. A total of 16 patients showed some form of malabsorption. Pathological liver function tests were obtained in five cases, and signs of pancreatitis were observed in one patient.

Many diseases of the gastro-intestinal tract, e.g. salmonellosis, shigellosis, ulcerous colitis, regional enteritis and Whipple's disease are complicated by arthritis. Various systemic manifestations, such as changes of the heart, lungs, kidneys, nervous and vascular systems, have been described in rheumatoid arthritis (RA). The gastro-intestinal disturbances have attracted relatively little interest, although many RA patients complain of symptoms of this kind.

In patients with RA the frequency of gastric ulcer has been found to be double the frequency in the general population (7). Toivonen et al. (15) described two patients with systemic connective tissue disorders, malabsorption syndrome and sprue-like changes in the intestinal mucosa, and Siurala et al. (13) noted malabsorption in seven of 71 patients with different collagen diseases.

The liver and pancreatic functions in RA have also attracted relatively little interest. The well-known syndrome of lupoid hepatitis is probably a manifestation of systemic lupus erythematosus (SLE). Some cases of pancreatitis associated with RA have been reported, but in these the condition has been attributed to the steroid treatment administered (11).

So far no report has been published on patients with RA investigated for malabsorption. In the present RA series the intestinal, liver and pancreatic functions were studied.

MATERIAL AND METHODS

The series consists of 22 patients with RA and two with active rheumatoid spondylitis treated at the Fourth Department of Medicine, Helsinki University Central Hospital, and/or the Rheumatism Foundation Hospital, Heinola. Of these, 15 were females and nine males. Their age ranged from 20 to 73 years. The duration of the disease was less than five years in 14 cases, above five years in the remainder. All patients with RA complied with the criteria for definite classical severe rheumatoid arthritis. At the time of investigation all patients had received treatment in the form of salicylates, phenylbutazone, indometacine or corticosteroids. Some patients had previously been treated with gold.

Glucose-galactose and lactose tolerance tests were performed in all cases. The former consisted of 25 g glucose + 25 g galactose in 200 ml of water administered on fasting stomach. The latter consisted of 50 g lactose in 200 ml of water. Blood sugar was determined in an auto-analyzer (glucose oxidase method) prior to the tolerance test and 15 30 45 60 and 90 min after the patient had taken the sugar solution. A rise in blood sugar under 25 mg/100 ml was considered pathological.

Intestinal biopsy was performed using Bolt capsule, which is modification of the Crosby-Kugler capsule (12). The capsule was brought down to the first loop of jejunum and the position checked by X-ray. The

Table I. *Disaccharidase activity at the first loop of jejunum in nine patients with glucose-galactose tolerance curves > 25 mg/100 ml and lactose tolerance curves < 25 mg/100 ml. Intestinal mucosa not atrophic in any case but nos. 5 6 8 and 9 showed perivascular amyloidosis*

Case no	Maltase	Disaccharidase activities (units/g protein) Saccharase	Palatinase	Lactase	Cellobiase	Lactase-to-saccharase ratio
1	579	176	40	62	13	0.35
2	456	126	30	7.9	1 1	0.06
3	107	30	7 1	25	7.8	0.80
4	400	125	31	7 8	0.9	0.06
5	340	96	23	54	11	0.50
6	315	76	18	3.5	—	0.04
7	173	47	9.9	12	2.8	0.25
8	439	158	32	4.9	0 5	0 03
9	355	99	24	3 0	0 5	0.02

specimen was divided into two parts. One was fixed in 10% neutral formalin, embedded in paraffin, sectioned at 5 μ and stained with haematoxylin-eosin and van Gieson. The congo red, methyl violet and thioflavine T methods were used to demonstrate amyloid. The other part of the specimen was immediately wrapped in parafilm and deep-frozen. Later the disaccharidase activities (lactase, cellobiase, maltase, saccharase and palatinase) were determined per g of protein according to the method described by Dahlqvist (7) and modified by Launiala et al. (6) The disaccharidase activity is expressed as units g protein. By the method used, the lower limit for the normal lactase activity is 15 units and the lower limit of the normal lactase-to-saccharase ratio is 0.3 (Determinations performed at the Laboratory of the Foundation for Pediatric Research, Helsinki.)

The *d*-xylose tolerance was tested in 19 cases, the concentration in 5-hour urine being determined after an oral dose of 25 g. Pathological values were taken into consideration only if the volume of urine exceeded 500 ml. An excretion of less than 5 g was considered pathological. Vitamin A tolerance test was performed in 23 cases by determination of the concentration in the blood before and four hours after the administration of 350,000 IU vitamin A. The B_{12} level in the serum was determined in 14 cases by microbiological assay with Euglena gracilis (1), and the folic acid level was determined in 14 cases by the method of Girdwood (4). The 48-hour vitamin B_{12} absorption was tested by the method of Schilling et al. (10) using a dose of 1 μg of ^{60}Co-labelled vitamin B_{12} and a single flushing dose of 1 mg unlabelled vitamin B_{12} two hours later. Serum iron was determined in all cases. In six cases pancreatic function was estimated on the basis of the quality and quantity of the excretion through a duodenal tube after secretin stimulation. In 19 cases some or all of the following tests were performed: glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactic dehydrogenase (LDH) and its isoenzymes, bromsulphalein retention (BSR) after 45 min, alkaline phosphatase and serum bilirubin. Needle biopsy of the liver was performed in three cases.

RESULTS

Small intestine

Ten of the 24 patients had a flat lactose tolerance curve and a normal glucose-galactose tolerance curve. Intestinal biopsy was performed in nine cases. In six of these the lactase concentration was lowered, while the other enzyme activities were normal (Table I). Mucosal atrophy was not observed, but four patients showed amyloidosis with a perivascular distribution in the lamina propria (Fig. 1). Two patients showed normal lactase activity in spite of a flat lactose tolerance curve. In one patient the activities of all enzymes were low in spite of a normal mucosa.

In the remaining 14 cases both the lactose and the glucose-galactose tolerance curves were normal. Intestinal biopsy performed for the sake of comparison in three of these cases showed a normal mucosa and normal enzymatic activities.

Serum iron was low in 10 patients. The *d*-xylose tolerance test was pathological in seven, and so was the vitamin A tolerance test in nine patients. The folic acid level in blood was lowered in five cases (Table II). One of these patients also showed a low B_{12} level but the Schilling test was normal.

Pancreas

In five cases pancreatic function was studied by duodenal intubation and secretin stimulation. According to Siurala et al. (13) 70 mEq/l is the lower limit for the normal bicarbonate concentration. A pathological value was noted in one patient, who showed occasional abdominal pain,

typical of pancreatic involvement. The glucose tolerance test was normal, but the lactose toler ance test was flat. Serum lipase and amylase were studied in 13 patients, who all had normal values.

Liver

Liver function was studied in 19 patients on the basis of some or all of the following tests: GOT GPT LDH isoenzymes, alkaline phosphatase, serum bilirubin and BSR (Table II). Five patients had one or more pathological tests. Two patients had increased GOT and GPT values and a pathological bromsulphalein test (over 10% in 45 min). In both cases the LDH isoenzymes exhibited an increase of the slow fraction, while the total LDH activity was normal. On the basis of liver biopsy performed in one of these cases a pathoanatomical diagnosis of cholangiolitis was made. In one patient with increased GPT values and a pathological bromsulphalein test a biopsy specimen of the liver was normal. In another two patients only the bromsulphalein test was pathological.

In spite of repeated investigations, LE cells could not be demonstrated in any of the patients with pathological liver function tests. One patient exhibited positive antibodies to nucleic components as demonstrated by an immunofluorescent technique. Biliary tract surgery had been performed in only one case, in which cholegraphy was normal. In the remainder of cases cholecystography was also normal, except in one patient who had gallstones.



Fig. 1. Jejunal mucosa from patient (no. 6) with selective lactose malabsorption. Villi normal in appearance, but perivascular amyloid infiltration as demonstrated (green fluorescence in ultraviolet light with thioflavin T metachromasia with methyl violet and red staining ith congo red) HE, 100

DISCUSSION

In regard to the etiology of RA, the most plausible theory so far advanced seems to be that of an infectious origin. The intestinal bacterial flora has attracted interest in this connection. Enterococci (14) and chlostridia (9) have been studied from the standpoint of a possibl cor relation. If an association exists, intestinal disturbances may develop at the same time as arthritis.

In six of 22 patients with RA selective lactose malabsorption was observed. Two of these patients had noticed that milk produced symptoms in the form of diarrhoea and abdominal pain, while four were not aware of the association. However it should be borne in mind that lactose intolerance is not rare. Jussila et al. (5) noted lactose intolerance in about 15% of normal subjects in a Finnish population. Hence it is difficult to decide whether the present observations are due to chance or reflect a true association between RA and this form of malabsorption. Newcomer and McGill (8) detected lactose intolerance in six out of 100 healthy persons. It is noteworthy however that in two patients of the present series the lactose toler ance curve was normalized when the RA reached more quiescent stage. This may be evidence of connection between the active phase of the disease and the gastro-intestinal disturbance The same patients also showed a disturbed liver func tion with elevated GOT and GPT values and bromsulphalein retention of 6 and 19.5% respectively in 45 min during the acute phase of RA. Liver function was also normalized when a more quiescent stage was attained by trea

Table II *Results of tolerance and liver function tests and intestinal biopsy in 22 patients with RA and in two patients (cases 2 and 15) with rheumatoid spondylitis*

Case no	Lactose tolerance test	Glucose-galactose tolerance test	d-xylose tolerance test	Vitamin A tolerance test	Folic acid (μg/ml)	Vitamin B_{12} (pg/ml)
1	P	N		N		
2	P	N	7 1	P	3.5	300
3	P	N	6.8	N	1.4	200
4	P	N		N	3.5	265
5	P	N	3.4	P		
6	P	N	2.7	N	6 8	310
7	P	N	6.7	P	9 0	300
8	P	N			2.7	460
9	P	N	2.1	P	3.0	140
10	P	N		P	5.3	240
11	N	N	1 7	P	3 1	170
12	N	N	4.3	N		
13	N	N	8.4	P		
14	N	N	7 0	N	6.8	145
15	N	N	9.3	N	3.3	420
16	N	N	4.6	N	1.6	240
17	N	N	6.4	P	2.7	130
18	N	N	6.3	P		
19	N	N	6.4	N		
20	N	N	6.2	N		
21	N	N	6.1	N		
22	N	N		N		
23	N	N	5.5	N	2.9	360
24	N	N	2.2	N		

N normal. P pathologic. A, amyloidosis.

this connection one case may be described in detail.

Case 9

A woman, 67 who in 1968 developed severe rheumatoid arthritis with involvement f the majority of synovial joints during the first year. In the summer of 1969 lactose tolerance was still normal. Half a year later in Dec. 1969 the lactose and vitami A tolerance tests were markedly pathological, and intestinal amyloidosis was demonstrated. At the same time antinuclear antibodies were found in serum, and proteinuria and haematuria were observed. In this case exacerbation of the basic disease was definitely associated with malabsorption.

Follow-up examinations of another two of the patients with lactose malabsorption showed no change of the condition.

Since LE cells could not be demonstrated in any of the five patients with liver function disturbance, the possibility of lupoid hepatitis seems to be ruled out. One patient (no 21) had gall stones, but no subjective symptoms from the biliary tract. In this case it is impossible to decide whether biliary tract disease was the cause of the liver function disturbance. In the remaining four patients no biliary tract disease was observed One of these (no. .4) showed only a pathological bromsulphalein retention. She also had renal amyloidosis, and it should be borne in mind that pathological bromsulphalein retention has been described in connection with amyloidosis (3) In regard to the remaining three patients, who all showed pathological enzyme values and a pathological bromsulphalein retention, the rheumatoid arthritis was the only potential cause that could be demonstrated. Liver biopsy performed in two of these cases revealed signs of cholangiolitis in one and vacuolized cells in the other It does not seem probable that the treatment given had caused the liver damage One of these patients showed improvement when the treatment was instituted, the other when prednisone was added to the regimen. The third patient was throughout treated with mofebutazone, which is known to be liver-toxic. In this case the liver function was normalized during hospitalization.

Amyloid was observed in five patients: In the

Serum iron (μg/100 ml)	GOT	GPT	LDH	LDH isoenzymes	BSR	Intestinal mucosa	Disaccharidase activity
112	19	22			4	N	N
92	14	15	180	N	4.1	N	P
22	54	53	190	P	26	N	N
26	12	15	320	N	11.5	N	P
41	16	17	240	N	5	A	N
23	8	7			3	A	P
25	16	69	190		19.5	N	P
32	10	18	180	N	5	A	P
19	19					A	P
42	14	25	180				
31	10	5	170	N	2.5	N	N
54	14	14					
19	13	15			5		
26	7	4			7.5		
31	14	7	140	N	5		
88	4	7	150	N	3	N	N
31	10						
37	14				5	N	N
40	14	10	160	N	3.5		
34	17						
42	61	114	130	P	14		
46	614	768					
112							
13	11				18		

intestine in four and in the kidneys in one. Three of the patients with intestinal amyloidosis also showed selective lactase deficiency. The other had a flat lactose tolerance curve, while the mucosal enzyme activity was normal. The amyloid distribution was perivascular.

Only one patient showed signs of pancreatic insufficiency. She had a history of severe abdominal pain and intermittent diarrhoea for some length of time. In this case, too, selective lactose malabsorption and liver function disturbance were observed. Pancreatitis has been reported in connection with cortisone treatment (11), but this patient had not received cortisone. No signs of biliary tract disease were discovered, and the condition was not due to alcoholic abuse.

Of the patients with various collagen diseases described by Siurala et al. (13), almost half had one or more pathological tolerance tests. The intestinal mucosa was atrophic in seven of 71 patients. In the present series mucosal atrophy was not observed, but selective lactose deficiency and amyloidosis were noted. Over half of the patients in this series, which predominantly consists of RA cases, had one or more pathological tolerance tests.

It is of course difficult to decide to what extent the antirheumatoid treatment may have caused the disturbances of the gastro-intestinal, liver and pancreatic functions, and to what extent these may have been due to the rheumatoid arthritis. However, the fact that in many cases these disturbances disappeared or were mitigated during treatment seems to indicate that the therapy may be excluded as causative factor and that the gastro-intestinal disturbances were associated with the basic disease.

The present results show that changes of the gastro-intestinal tract associated with RA merit greater attention than has hitherto been paid to them.

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NEPHRONO-PHTHISIS

A Uremic Disease with Hypotonic Urine

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Abstract. Eighteen index cases of nephrono-phthisis have been found and their first-degree relatives, and in some instances also more remote relatives, have been examined to elucidate the transmission and the variability of the clinical expression of the disease. This examination included analysis of available clinical records, renal function tests in living individuals and histological re-evaluation of available kidney specimens. The findings suggest that: 1) the disease may appear in succeeding generations; there is some evidence in favor of dominant autosomal inheritance, although no definite conclusions are drawn concerning the type of transmission; 2) adult patients may exhibit a disease pattern the features of which are difficult to identify both from the clinical and morphological points of view.

It is re-emphasized that "medullary cystic disease" and "familial juvenile nephrono-phthisis" constitute one and the same disease.

In 1951 Fanconi et al. (5) presented a detailed description of a chronic renal disease which appeared in children in certain families and was given the name *familiäre juvenile Nephronophthise*. Numerous reports of this disease have since appeared, particularly in the European but also in the American literature (1, 2, 6, 8, 9, 10, 11, 12, 13, 15, 21), and its clinical and morphological characteristics have gradually been clarified. The main clinical features consist of hypotonic polyuria, uremia, often salt loss, a normal blood pressure and negative urinary findings. Histologically Fanconi et al. found a diffuse atrophy of the nephrons leading to severe and uniform contraction of both kidneys. It has later been shown that both atrophic and hyperplastic tubules occur (11) and that cysts may appear in the renal medulla (1, 6, 11, 15).

In 1962 Strauss presented 18 cases with the above clinical picture and with cystic lesions in the renal medulla as the most conspicuous morphologic feature. This disorder he called *cystic disease of the renal medulla*. In six adults in which he encountered this morphologic lesion the leading clinical feature of the children, the polyuria, was lacking (19). On the other hand proteinuria and/or arterial hypertension was recorded in some of the adult patients. It has recently been shown that this "atypical" clinical picture may appear also in adolescent patients with morphological lesions typical of nephronophthisis (12).

On the basis of the above experiences we have previously suggested that nephrono-phthisis and cystic disease of the renal medulla may in fact be identical (12) and this view has been advanced also in the American literature (15, 20). A typical form with hypotonic polyuria and an atypical form without this feature may appear both in young patients and adults (12). These features are further elucidated in the present family study, the purpose of which is:

1. to present evidence that the disease may appear in succeeding generations,

2. to suggest that adult patients, and possibly also juvenile patients, may exhibit a disease pattern the features of which are difficult to identify both from clinical and histological points of view and

3. to discuss the question of nomenclature with regard to our opinion that nephrono-phthisis and

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Table I. *Criteria for diagnosis of nephrono-phthisis in index cases, siblings and one other juvenile relatives*

ND = not done

Family	Case	Family members	Typical clinical picture	Typical histologic findings	Ref. no.	Case no
1	C	Index case	Yes	ND	6	1
	E	Brother	N	Yes	Unpubl.	
2	C	Brother	Yes	Yes	Unpubl.	
	D	Index case	Yes	Yes	6	
3	C	Index case	Yes	Yes	6	3
4	C	Index case	Yes	Yes	6	4
5	C	Sister	Yes	ND	Unpubl.	
	D	Index case	Yes	ND	6	5
6	D	Index case	Yes	ND	6	7
7	C	Index case	Yes	Yes	10	1
8	C	Index case	Yes	Yes	Unpubl.	
	X	Mother[a] cousin	Yes	Yes	Unpubl.	
9	C	Index case	Yes	ND	3	k
	D	Sister	Yes	Yes	3	U
10	C	Brother	Yes	Yes	3	E
	E	Index case	Yes	Yes	3	M
11	D	Index case	No	Yes	12	1
12	E	Index case	Yes	ND Alive	Unpubl.	
13	C	Brother	Yes	ND	Unpubl.	
	D	Index case	N	Yes	12	2
14	G	Index case[a]	No	No Alive	Unpubl.	
15	C	Index case	Yes	Yes. Alive	Unpubl.	
	E	Brother	Yes	Possibly[b] Alive	Unpubl.	
16	F	Index case	Yes	Yes	Unpubl.	
17	C	Index case	Yes	Yes. Alive	Unpubl.	
18	D	Index case	Yes	ND Alive	Unpubl.	

[a] See text.
[b] Biopsy findings not contradictory to nephrono-phthisis.

cystic disease of the renal medulla constitute one disease.

MATERIAL AND METHODS

The material consists of all families in Sweden in which cases of nephrono-phthisis came to our knowledge before January 1, 1967 and in which family investigation could be performed. No systematic search for cases of nephrono-phthisis was made. The number of families therefore does not reflect the incidence of the disease in this country.

Index cases

The eighteen families were all discovered through the occurrence of *nephrono-phthisis in one member—the index case.* These cases have either been under the care of one of us or brought to our attention by colleagues, or have they been published previously. Clinical data and available kidney specimens have been re-examined. In 11 of the index cases the diagnosis has been based on the wellknown clinical features (1 ... 5 6 8, 9 10, 11, 13 15 ...) and/or on typical histologic findings (1, 5, 6, 11); in six of the cases it has been based on clinical data only (Table I). In one index case (family 14) nephrono-phthisis was suspected only on clinical grounds, and renal biopsy was inconclusive. This family has been briefly discussed previously (12). It is included in the present study since the index case may well be a case of atypical nephrono-phthisis and family examination has revealed that the mother died at the age of 40 years with clinical features and histologic renal lesions typical of nephrono-phthisis.

Family investigation

In each family a thorough history with regard to symptoms of renal disease in siblings and parents was obtained, and available histologic specimens in these family members were re-examined. In addition, the hospital records of all relatives in whom renal insufficiency was known were scrutinized and available histologic specimens re-examined.

In fifteen parents and nine siblings without known renal disease a full clinical examination program could be performed. This included physical examination and the following laboratory tests: "true" C_{cr}, renal concentration capacity, serum electrolytes (including calcium and phosphate), red cell excretion during 12 h, excretion of albumin, pus cells and casts, hemoglobin and 1 h sedimentation rate. Urine examinations were performed on morning specimens. All blood examina-

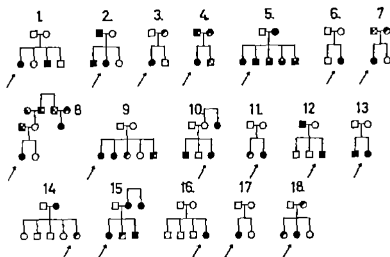


Fig. 1 Pedigree chart of 18 families with cases of nephrono-phthisis. Severe renal disease in two successive generations occurred in five families. In three more families renal disease was highly suspected in the parent generation of the index cases. In no family was there any suspicion that renal disease had affected both parents.

♂ ♀
□ ○ N renal disease.
■ ● Proved or probable nephrono-phthisis.
◪ ◐ Impaired renal function (proved or possible).
⊡ ⊕ History negative. No laboratory investigation.
↗ Index case.

A B C D E F G — Chart showing the principles according to which the family members have been designated.

tions were performed on fasting morning specimens. C_{cr} was determined by means of two 12-hour overnight urine collections; concentration capacity was estimated after pitressin administration combined with about 16 h thirst. In five more families (families 14, 15 16, 17 and 18) only part of this program was performed, usually serum creatinine, concentration capacity and urine examination.

RESULTS

The families of the index cases—and in three instances more distant relatives—are shown in Fig. 1 In Fig. 2 the results of the investigation of C_{cr} and concentration capacity in parents and siblings are plotted. Those with an obvious nephrono-phthisis are excluded, as are the members of the five families in which only part of the examination program was performed. All except case 7 B thought themselves to be healthy

Clinical Observations

Parent generation

Six adults in the parent generation died of renal insufficiency two fathers, two mothers and two aunts. Relevant clinical data and results of laboratory investigations are summarized in Tables II and III. Although all of these six cases were classified as chronic glomerulonephritis at the time of death, their renal disease had some notable features that make this diagnosis questionable. Thus there was a tendency to produce low concentrated urine in amounts which, in for example cases 2 A and 12 A, were remarkably large with regard to the marked reduction of C_{cr}. Furthermore, proteinuria was absent or slight, and abnormal excretion of red and white cells was not seen until shortly before death. Casts were never observed in the urine. Salt loss occurred sooner or later in all patients in whom serum electrolyte determinations were made. Another notable feature was glucosuria or decreased glucose tolerance (5 patients). These features are very reminiscent of those present in the diseased children.

Of the living parents in whom the full examination program was performed, two had a markedly lowered C_{cr}, one of them also with a low concentration capacity (Fig. 2). Four more parents showed borderline values for concentration capacity or C_{cr} Case 3 B had a true

rather young age in several siblings of one of the parents (case 2 A) and in several first cousins of another parent (case 13 B). We have been able to objectivize this in two instances; the other individuals have not been possible to trace.

Case 8 X, although not adult, should also be mentioned. She was first cousin of the mother of index case 8 C. She died at the age of 11 years with a classical clinical and histological picture of nephrono-phthisis.

Siblings

There were 34 siblings of the 18 index cases. Three of these siblings were unavailable for investigation (5 E, F and G) and two others refused (4 D, 15 D). The latter (15 D) was said to have uremia.

Seven of the siblings were cases of classical nephrono-phthisis (Fig. 1). Two others (3 E and 11 C (27 years)) had a low C_{cr} (Fig. 2). Of those without known renal disease who were incompletely investigated one sister (18 C) of case 18 D concentrated to a specific gravity of 1.021 during overnight thirst. Like her mother who is mentioned above, and the diseased brother, she had a history of intermittent glucosuria for many years. Serum creatinine was not determined.

The fallacies of an investigation like the present one is illustrated by the following incident. The parents in family 1 admitted investigation of siblings 1 D and 1 F but refused in sibling 1 E, saying he was healthy. Some months later he was admitted to hospital and died of uremia. He proved to have a history of nephrono-phthisis of long duration, and the histological findings were typical of that disease (see below).

Fig. 2 ... capacity ... phthisis ... and con- ... 3 B ... healthy ... highly ... The high ... be faulty ... the fact that the specific gravity urine ... included ... according ... Methods, after overnight ... concentrated, however only to a specific ... 1.016 and 1.021

... of 2.0 mg per 100 ml and case ... of 1.2. Case 3 B excreted moderate ... white and red cells. She had a disturb- ... large. Otherwise urinary findings and ... were normal in both patients.

Of those who underwent a less complete investigation the mother of case 18 D concentrated her urine to a specific gravity of 1.016 during overnight thirst. She had intermittent glucosuria. Serum creatinine was not determined.

In this connection it might be mentioned that there was a history of hypertension or stroke at

Morphological Examination

Parent generation

Paraffin blocks of the kidneys were included in the autopsy material of four of six cases in the parent generation in which death had resulted from renal disease and uremia. The following main lesions were observed in these four cases (cases 2 A, 12 A, 14 B and 15 F).

Case 2 A. The kidneys were pale and reduced in size (weight 115 g each). There were moderate hypertensive vascular changes in the kidneys. A varying degree of glomerular degeneration and

Table II. *Anamnestic and clinical findings in adult relatives who died of renal insufficiency and belonged to the generation immediately preceding the probands*

	Case 2 A	Case 10 F	Case 12 A	Case 14 B	Case 15 B	Case 15 E
Age at first admission	53	49	45	37	33	51
Age at death	54	52	46	40	35	51
Admitting complaint	Acute heart insufficiency	Headache. Tired	Calf cramps. Headache	None. Hypertension at routine check up	Tired. Skeletal pains	Tired, slight hemiplegia
Subjective polyuria	Not distressing	None	Not distressing	Slight	Slight	None
Polydipsia	No	No	N	No	Slight	None
Renal history	Yes	Yes	Proteinuria at 11 years of age	Proteinuria after 5th delivery	Repeated urinary tract infections	Yes
Physical activity normal	Yes, until shortly before	No, low for ½ year	Yes	Yes	Yes, almost	No
Tetany	No	No	Yes?	No	N	No
Blood pressure	230/165	140/90	210/120	210/140	140/80	40/140
Eyegrounds	Hypertensive changes	Normal	Normal	Hypertensive changes	ND	Hypertensive changes
Kidneys on X-ray	Probably normal	ND	ND	Small	Small	ND
Family history positive	Yes	Yes	Yes	Yes	Yes	Yes
Preceding generation affected	No	No	Unknown	No	No	No
Same generation affected	Yes	N	Unknow	No	Yes	Yes
Following generation affected	Yes	Yes	Yes	Yes	Yes	Yes
Husband (de)	Nephrectomy because of renal tbc	Healthy	Healthy	Healthy	Healthy	—

fibrosis was present; some glomeruli were normal or hyperplastic. There was some degree of tubular atrophy. Some loops of Henle were slightly tortuous, with a slight thickening of the basement membranes. An irregular dilatation of tubules was noted but no clear cyst formation. There was no appreciable infiltration of inflammatory cells.

Case 12 A. The kidneys were small, contracted and equal in size (weight 55 g each). The histologic kidney lesion was very similar to that in case 2 A. Indeed, no major differences could be seen between these two cases.

Case 14 B. The kidneys were considerably contracted and equal in size (weight 35 g each). Extensive glomerular degeneration and fibrosis was present, some glomeruli were, however intact or hyperplastic. Apart from widespread tubular atrophy there was also tortuosity of loops of Henle and a moderate degree of basement membrane thickening of these tubules. Numerous cysts of varying sizes in the medulla were observed, no cysts were found in the cortex. There were moderate hypertensive vascular changes in the kidneys, and small foci of acute pyelonephritis.

Case 15 F. There were considerable hypertensive vascular changes in the kidneys with marked degree of arteriolosclerosis, arteriolonecrosis and interstitial hemorrhages. Most glomeruli were unaltered. There was no evidence of inflammation. No cyst formation or tubular tortuosity was observed.

Siblings

Material for histologic examination was available from five of the seven diseased siblings (1 E, 2 C, 9 D, 10 C and 15 C). The material of cases 9 D and 10 C was placed at our disposal by Dr C. Lundmark, Danderyd Hospital, Danderyd, who has reported these cases together with Hackzell. The other three cases have not been reported previously.

In cases 1 E, 2 C, 9 D and 10 C material was obtained at autopsy and the kidney lesion was

Table III. *Laboratory findings in adult relatives who died of renal insufficiency and belonged to the generation immediately preceding the probands*

	Case 2 A	Case 10 F	Case 12 A	Case 14 B	Case 15 B	Case 15 E
Urine						
24 h urine vol. in l and spec. grav	0.8–2.6 usually 1.5 1.011 1.015	1.5–2.3 ND	1 1 –1 1.003–1.005	1.5–3.3 1.005–1.010	1.3–3.5 usually 1.5–2.0 1.004–1.012	Below 1 1.005–1.019
Max. urine conc. cap	1.015	ND	ND	1.010	ND	ND
Max. urine dil. cap.	1.005	ND	ND	1.003	ND	ND
Protein ‰	0.2 1.8	0–0.6	0 7–1.8	0 1 1.5	Neg.–slightly pos.	Neg.–4
Erythrocytes, white cells, cylinders	Nothing abnormal[a]	Nothing abnormal	Nothing abnormal[a]	Nothing abnormal[a]	Occasional pyuria.[b] No red cells or cylinders	Erythroc. none–15/s in-al field. WBC constantly present. No casts
Glucose	On one occasion 0.2 %	Neg.	Intermittently present[c]	Neg.	Neg.[d]	Intermittently present[c]
Blood						
N.P.N. or creatinine mg 100 ml	49–221 2.5–8.0	90–180 —	66–228 8 4–18 6	135–240 —	134–368 —	46–104 —
Clearance	C_{cr} 33–7 ml/min	C_{cr} 8 1/24 h	C_{cr} 13 1/24 h	C_{cr} 55 1/24 h	C_{cr} 8–9 ml/min	ND
Cholesterol, mg 100 ml	290–390	ND	279	ND	229	ND
Total lipid	570–960	ND	770	ND	ND	ND
Protein	7 1	ND	5.8	ND	6.9–9 7[e]	ND
Sodium	138–127	ND	146–121	179	128	ND[f]
Potassium	4 1–4 9	ND	5.5–5 8	4.5–6.0	5–6.5	ND
Chlorides	102–88	69	106–86	ND	90–66	ND[f]
Calcium	8 6–7	ND	6.3–7 1	ND	About 10	ND
Phosphate	7 0–10 1	ND	ND	ND	7 3–10.7	ND

[a] White and red cells shortly before death
[b] Culture negative
[c] Blood sugar case 2 A, 100 mg 100 ml case 1 A, 85–117 mg 100 ml, case 15 E, 113 mg 100 ml.
[d] *Glucose tolerance decreased.*
[e] Probably due to dehydration.
[f] Sodium chloride 94 mEq l two days before death.

found to be typical of nephrono-phthisis (Table I) This included severe and diffuse contraction of the kidneys with atrophy and focal hyperplasia of the tubules, considerable thickening of the basement membranes of the loops of Henle and distal tubules, and widespread glomerular damage leaving some glomeruli intact or hyperplastic. In three of the cases (1 E, 2 C and 10 C) numerous cysts were found in the medulla. In the fifth sibling (15 C) material was obtained by percutaneous renal biopsy This material was sparse and derived from the cortico-medullary junction. On the medullary side there was considerable atrophy *of some tubules with thickening* of the tubular basement membranes occasional tubules were markedly dilated. The interstitial tissue showed contraction without evidence of inflammatory cell infiltration. On the cortical side two glomeruli were included in the specimen. These showed *no appreciable alterations*. In summary the morphologic picture in this case may be regarded as suggestive but not diagnostic of nephrono-phthisis.

Genetic-statistical Analysis

Maternal age

If a disorder stems wholly from genetic factors, affected persons should not differ from people as a whole in parental age. To see whether the present series deviated from normal in maternal age, *we compared the patients with a control* series selected from the general population. This series consisted of women from the general Swedish population who produced children in the same year as the index cases were born.

Table IV shows the maternal age at birth for the index cases and the control series (expected values). The mothers were 29.8 years old on the average when the patients were born, and 29.3 years old when the controls were born. This is a nonsignificant difference.

Geographical distribution and consanguinity

When our 18 index cases were grouped according to the county in which they were born, it was shown that the birthplaces were spread throughout Sweden. Data for calculation of the frequency of consanguineous marriages were gathered from the parish records. Information on birth registration data were available in four generations back for both parents and their ancestors of 16 index cases. By this genealogical study we could in all pedigrees exclude consanguineous relations up to fifth-degree (e.g. first cousins once removed).

Morbidity among siblings

Our introduction to the families exhibiting nephrono-phthisis occurred because of an affected member who happened to come for medical attention. Quite aware of the fact that the data were incomplete, we decided on the basis of the comparatively large number of families to test our family data by the Apert–Bernstein method. This method, variously also called the "direct method" or the "a priori method" endeavors to determine whether the observed ratio agrees with the a priori expectation, if it does, it is assumed that the observed ratio is of the kind being tested for. The test of agreement of the data with the hypothesis consists of a comparison

Table IV *Mothers of 18 individuals with nephrono-phthisis by age at birth of the affected child*

Maternal age (y)	Observed no. of mothers	Expected no. of mothers
20	—	0.99
20–25	5	4.23
25–30	3	5.10
30–35	6	4.08
35–40	4	2.50
40–45	—	1.00
45–	—	0.10
Total no.	18	18.00
Mean age	29.78	29.32

Table V *Analysis of 18 sibships with one more case of nephrono-phthisis by Apert Bernstein method*

Dominant mode of inheritance postulated

Family size	No. of families	No. affected observed	No. affected expected	Variance
2	8	10	10.664	1.776
3	5	10	8.575	2.450
4	2	3	4.268	1.564
5	3	6	7.743	3.246
Total	18	29	31.250	9.036

of the observed and expected total number of affected members with the standard deviation of the latter

Table V gives a comparison between the numbers of affected siblings observed and the numbers which would be expected with a dominant mode of inheritance according to the Apert–Berstein method. We have here included the five siblings designed as probably affected, whereas the five siblings of whom we lacked data were considered healthy

Assuming a dominant inheritance the difference between the total number of affected siblings observed (29) and the total number expected (31.25 ± 3.01) is less than one standard deviation. This is clearly a nonsignificant difference. If we assume a recessive inheritance, however the difference between the observed (29) and expected (23.47 ± 2.22) numbers approximately amounts to 2 $/_{2}$ standard deviations.

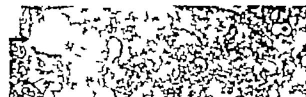
DISCUSSION

The main question raised by this investigation is whether fatal nephrono-phthisis can appear in succeeding generations. If so, this should be a strong challenge to the common conception of this disease as inherited through recessive autosomal gene (5 13). If *both* typical clinical findings *and* demonstration of the typical histologic changes are required for diagnosis, the present material contained only one example of the ppearance of the disease in succeeding generations (family 8) and no unequivocal example of its appearance in both parent and child. There are, however several circumstantial facts suggesting a direct transmission from parent to child.


a










d

In families 2, 10 and 15 in which the index cases (and/or siblings) showed all three diagnostic criteria (typical clinical picture, typical histology and diseased siblings), *four* individuals in the parent generation died of a renal disease (? A, 10 F 15 B and 15 E). The clinical data of these patients are included in Tables II and III.

From Table III it can be seen that in case 2 A, in which a full investigation of renal function was performed, the laboratory findings are non-specific but similar to those obtained in cases of typical nephrono-phthisis. The vascular contraction of the kidney was, however too advanced to permit of any detailed morphologic analysis, but glomerulonephritis and pyelonephritis could almost certainly be ruled out.

In family 10 the aunt had a uremic disease reminiscent of nephrono-phthisis: in particular the normal urine findings and the low serum chloride concentration are suggestive. No post mortem examination was performed.

In family 15 the mother showed a typical clinical pattern, the evaluation of which is however obscured by renal osteodystrophy and metastatic calcifications. This patient has earlier been reported as case of secondary hyperparathyreoidism (16) Notable in the autopsy record of this patient is the mention of cysts in the contracted kidney parenchyma (4). Specimens for histologic investigation were unfortunately not available.

Fig 3 (*a*) Section from the renal cortex of case 12 A showing atrophy of the tubules and interstitial fibrosis. One glomerulus appears normal (*bottom*) whereas second glomerulus (*top*) shows considerable thickening of Bowman capsule and extensive adhesions between tuft and capsule. Hematoxylin-eosin, 90.
(*b*) Section from the renal cortex of case 2 D showing atrophy of the tubules and interstitial fibrosis. Some glomeruli are normal, whereas other glomeruli show various degrees of degeneration with thickening of Bowman's capsule. Some glomeruli are almost converted to fibrous scars. Hematoxylin-eosin, 90.
(*c*) Section from the renal medulla of case 1. A showing atrophy of the tubules and interstitial fibrosis. There is irregular tubular dilatation its formation of small cyst-like spaces (*arrows*). Other tubules are markedly tortuous (*left*). Hematoxylin-eosin, 35
(*d*) Section from the renal medulla of case D showing alterations typical of nephrono-phthisis. There is interstitial fibrosis and many tubules are atrophic. Other tubules are highly tortuous. Cyst formation is prominent. Hematoxylin-eosin, 35

The aunt in this family (15 F) also showed severe renal disease with uremia, hypertension and advanced arteriolosclerosis in the kidneys.

The index case in family 12 has a clinically typical disease starting at the age of one year He is now ten. No renal biopsy has been performed. His father (1. A) died of uremia at the age of 46 The clinical picture of his disease has some features in common with nephrono-phthisis (Table II) just as have the fairly uncharacteristic kidney lesions. These lesions were very similar to those observed in case 2 A (see Results). In Fig. 3 the histologic picture of the kidney of case 12 A is compared with that of the daughter of case 2 A (? D), in which nephrono-phthisis was proven.

As to the type of inheritance the following findings may speak in favor of an autosomal dominant transmission and gainst a recessive one:

(*a*) the high morbidity risk observed among siblings of both sexes:

(*b*) the absence in our series of consanguinity among parents of affected individuals:

(*c*) the absence of evidence that in any family renal disease had affected *both* parents.

The last mentioned observation perhaps merits an explanation. We have earlier suggested that affected adults would be heterozygotes for a gene that in double doses leads to nephrono-phthisis (12). The presumption that parents of affected children would be carriers tallies at first glance with the repeatedly made observation that affected adults as a rule are much less severely affected than affected children. The very fact that in no family could we demonstrate or even suspect that a renal disorder had affected *both* parents must be a strong argument against such hypothesis. Furthermore, parenthood in itself practically excludes the possibility of a severe disease with invariably early onset. Variable expressivity is a very common attribute especially of dominant disorders such as dystrophia myotonica, Huntington's chorea and acute intermittent porphyria. Although the probability is thus in favor of a dominant autosomal transmission we wish to emphasize that our findings do not allow a definite conclusion about the way in which nephrono-phthisis is inherited, since it may occur in syndromes of different origins as sug

Table VI. *Comparison of clinical characteristics in juvenile and adult form of nephrono-phthisis*

Symptoms	Juvenile type	Adult type
Polydipsia	Yes	No
Hypotonic polyuria	Yes	No
Well retained dilution ability	Yes	Yes
Azotemia	Yes	Yes
Salt loss	Common	Common
Proteinuria	Insignificant	Often present 0–2‰
Urinary deposits	N	N
Hypertension	N	Yes

gested earlier by Mongeau and Worthen (15). For instance the role of external factors cannot be evaluated.

In conclusion, the clinico-pathologic examination of the material made a direct transmission of nephrono-phthisis from parent to child suspicious, but it could not be definitely established. A genetical analysis of the family data supported the idea of an autosomal dominant inheritance of the disease.

The probable direct mode of inheritance of nephrono-phthisis and the variations in the clinical picture that this disease may show as suggested by the findings in the present investigation, will be of importance in the management of the individual case. For eugenic advise a correct diagnosis is a prerequisite. Furthermore, treatment of certain types of proliferative glomerulonephritis with immunosuppressive compounds has given promising results (14). This treatment should probably not be given to patients with nephrono-phthisis when immunologic factors seem to play no part. Lastly if renal transplantation is considered, one must, in selecting renal donors, take into account the fact that nephrono-phthisis may manifest itself rather late in relatives of a juvenile patient. One may question whether a relative should ever be accepted as donor if it has not been clearly demonstrated that the gene comes from the other side of the family. Siblings should never be accepted as donors.

In the adult, nephrono-phthisis may be as easily diagnosed as in children (e.g. cases 14 B and 19 B). The present investigation suggests, however that the disease may appear in a form where hypertension is the dominating feature and polyuria not so prominent. In this form it may easily be mistaken for chronic nephritis or pyelonephritis, although the normal urinary findings, the often dilute urine and salt loss should alert the clinician. A comparison of the clinical features as they usually appear in the juvenile and the adult forms of the disease is given in Table VI. From the histologic point of view the problem is no less difficult since hypertensive vascular alterations will result in a contraction of the kidney parenchyma which may obscure any primary renal disease. A detailed analysis of the clinical and histologic difference between different types of chronic nephropathy has been given earlier (12).

Nephrono-phthisis has been considered very rare. There may be reason to re-evaluate this view. First, we have come across 18 families in Sweden, and one more has been presented by von Sydow and Ranström. Furthermore we have become aware of at least five more families in this country since January 1 1967. These families together constitute about 50% of all families hitherto reported. Four of the 24 families —all being unrelated—originate from a moderate-sized hospital where the staff some years ago became aware of the characteristic disease pattern through the occurrence of one typical case. Some of the adult and juvenile cases have been cared for both at university clinics and at specialized renal units under the diagnosis of chronic nephritis. This, together with the possibility that the histologic findings may be uncharacteristic in some adult cases, suggests that the disease pattern may easily be misinterpreted.

In early 1967 we suggested that what is known in the American literature as "medullary cystic disease of the kidney" and in the European as "familial juvenile nephrono-phthisis" are in fact identical diseases (12). Later this view was independently advanced by Mongeau and Worthen. It is further supported by observations on the heredity of so called cystic disease of the renal medulla. Recently Strauss and Sommers, having been presented with the original material of some of our cases, agreed that the diseases were clinically and morphologically indistinguishable, and therefore probably identical. As regards nomenclature, these authors preferred the term "medullary cystic disease" saying that "familial ju-

venile nephrono-phthisis" would be misleading since the disease is not restricted to the juvenile ages and single cases may appear in certain families. The former objection is certainly tenable. On the other hand, the present investigation has shown a familial trait of the disease, although it *may appear in its typical form in a single member*. Moreover the terminology of Strauss is unfortunate in that there are other diseases with cystic lesions in the renal medulla, e.g. medullary sponge kidney and cystic renal dysplasia. An even more important objection to the term "medullary cystic disease" is the fact that in many cases no medullary cysts are present (3, 13, 21).

The term "nephrono-phthisis" was applied to stress the degeneration of nephrons which is seen in the kidneys of the patients. This term, too, is in some measure misleading, since on the contrary there is often some hyperplasia of parts of the nephrons (11). The term, although somewhat inadequate, has, however, the advantage of being firmly associated with a distinct disease pattern in the European literature, and recently also in the American. In addition, the term will not lead to confusion with other disease entities. For reasons mentioned above, the term "juvenile" should be dropped in association with this disease. Since single *typical* instances of the disease may appear in certain families, the term "familial" should perhaps also be dropped for practical reasons. We therefore suggest that the disease should be termed simply "nephrono-phthisis" until a more appropriate denomination can be formulated on the basis of a better understanding of the pathogenetic mechanisms.

ACKNOWLEDGEMENTS

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APPENDIX

Case 2 A. PBA 100506

Father of proband case 2 D. Died of uremia and hypertension 54 years of age.

Genetic history. One son and one daughter died at the age of 6 and 1½ years, respectively. Both had hypo-

tonic polyuria, azotemia and normal urinary findings. The diagnosis of classical nephronophthisis was histologically ertified in both instances. The case of the daughter has been reported previously (2, 11). Four of eight siblings of the father are said to have severe hypertension.

Early history Noncontributive h renal or cardiac history

First signs of disease At the age of 53 years acute symptoms of left entricular failure.

First admission to hospital Aged 53, acutely hospitalized because of abov mentioned symptoms. Blood pressure .50 165 Eyegrounds Gr. IV hypertensive changes. Except for hypertension no signs of primary cardiac disease. The cardiac failure responded well to treatment. GFR was moderately reduced, 33 ml min; fell to 7 ml min one year later There was slight proteinuria but no urinary deposits, except for short time befor death. Urine culture negative. It is noticeable that 4 h urine volumes could amount to more than 1 although GFR was reduced to about 10 1/24 h. Dilution ability fairly well retained, concentration ability severly damaged. There was no history of polydipsia or distressing polyuria. Excretion of cathecholamines and their derivatives within normal limits. Serumproteins were normal. There as hypocalcemia and also tendency to lose salt.

Course After initial treatment in fairly good condition. Readmitted one year later with left ventricular failure. A tendency to lose salt had developed. The hypocalcemia as more pronounced. He died at the of 54 years. It should be noted that in the last days life there was erythrocyturia. Main findings are listed able II

Autopsy Significant alterations ere found in the cardiovascular system and the kidneys. The heart as considerably enlarged due to hypertrophy of the left ventricle all (heart eight 700 g) There as gener alized atheromatosis, hich more severe than nor mal for the age The lungs er markedly edematous (eights. left lung 1,000 g. right 1,050 g). The kidneys ere pale and equal in size, each eighing 115 g. The shape as normal. The surface as finely granular A few small, cortical cysts ere evident on the surface The cut surface displayed atrophy of the parenchyma, and blurred cortico-medullary border

Histologically the kidney showed reduced thickness of the cortical zone In this zone the arteries displayed marked degree of hyperplasia of the elastic wall lamellae, and the arterioles hyalinosis. The walls of some finer arteries ere markedly thickened due to cellular hyperplasia of the intima. The glomeruli varied in ppearance some appeared normal and slightly enlarged, others were partially or completely degener ated and fibrosed. There as some atrophy of the tubules. The medulla displayed slight increase in interstitial connective tissue. There ere no medullary cysts but numerous tubules ere irregularly dilated. Some descending and ascending limbs showed a slightly tortuous course. The basement membrane of these tubules was slightly thickened. There were no signs of nephrocalcinosis or pyelonephritis.

Case 10 F 03 07 04

Sister of mother of proband 10 E. Died of renal failure at the age of 52 years.

Genetic history Eight healthy brothers and sisters No know renal disease except for case 10 E.

Early history Up to the age of 45 subjectively well. N story of polyuria-polydipsia.

First signs of disease At the age of 45 severe and constant headache. At the age of 49 a blood pressure of 190/110 At this time there were no pathologic urinary findings and no anemia. NPN was not de termined.

First admission to hospital At 52 years, due to increasing weakness and vomiting. BP less than 140/80. Eyegrounds ere normal. Ther were no signs of primary cardiac disease. No edema. NPN was 135 mg/100 ml and increased to 180. Although she had a severe renal insufficiency ith C_{cr} of 8 1/24 h, urine volumes per 24 h were 1 3 1 with emarkably low specific gravity There was a trace of protein in the urine and nor mal sediment. She had severe anemia. Serum chlorides were low

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PACHYDERMOPERIOSTOSIS

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Abstract. The report concerns pachydermoperiostosis in two women, mother and daughter, a relationship which has never before been noted. Another unusual aspect is that the characteristic features of the condition were noted already at the birth of the daughter. Moreover both have had symptoms of disturbed gonadal function. The mother began menstruation at 20 years of age and had scanty menses. The daughter, now 30 years old, has still primary amenorrhoea and there is a low concentration of the oestrogens and FSH excretion in the urine.

The term pachydermoperiostosis was first used in 1948 by Vague (25) when he described 45 cases. Touraine et al. (21) had, however, listed the characteristics of the syndrome as early as 1935 under the name "pachydermie plicaturée avec pachypériostose des extrémités". The present conception of pachydermoperiostosis is therefore based upon their works. Earlier Bamberger (2) had described one case, and Marie (15) nine cases who were suffering from a closely related disease, hypertrophic osteoarthropathy. Marie called the condition "osteoarthropathie hyperthrophiante pneumonique" since he considered that the common factor was a lung disease. Among his cases without chronic lung disease are the brothers Wilhelm and Karl Hagner whose disease puzzled many famous clinical experts at the end of the 19th century (5, 8, 15, 20, 27).

DEFINITION

Pachydermia

Skin of the face, hands and feet grows coarse and thick, which causes deep furrows. The skin of the forehead forms transversal and vertical furrows. The nasolabial folds are marked. There is thickening of the soft parts of the face. The eyelids and superciliary arches may be marked. The scalp may be thickened and furrowed as in cutis verticis gyrata (12, 14). The skin is greasy and perspiring, above all on the hands. There is often blepharitis, acne and seborrhoea.

Pachyperiostosis

This may affect the whole skeleton, mainly the long bones of the extremities, metatarsal, metacarpal and phalangeal bones. The X-ray picture discloses cortical thickening as well as cortical exostosis. The sella turcica is of normal size.

The growth of the soft parts of face and extremities and the periostal ossification of hands and feet cause a more or less grotesque enlargement similar to acromegaly. The appearance has also caused some confusion with facies leonina in lepra. Furthermore there are clubbed fingers and "watch glass" fingernails. The disease is most common in men, but a few cases in women have been noted. The symptoms have been reported to begin in puberty and progress until the end of adolescence. The disease develops slowly and is neither painful nor related to a chronic lung disease in contrast to hypertrophic osteoarthropathy. The patients are in other respects healthy but cosmetic aspects and problems related to the enlarged hands and feet may become a serious challenge. From the first concept of the syndrome pachydermoperiostosis, Touraine et al. (21) distinguished three forms:

1. Complete form with pachydermia as well as pachyperiostosis but with varying intensity.
2. Incomplete form with pachydermia of extremities and face but not the scalp and with pachyperiostosis.
3. Forme fruste with pachydermia but without pachyperiostosis.

CASE REPORTS

Case 1

Born by Cesarean section in 1937. Weight 4100 g. Length 53 cm. Typical "acromegal" appearance, similar to the mother (case 2), was noted in the obstetric record. In childhood coarse facial features (Fig. 1) and increased growth of hair. At the age of 19 growth complete, height 169 cm. Size of shoes 6.

In 1956 examined because of primary amenorrhoea. External genitalia including clitoris normal. Laparotomy disclosed a rudimentary uterus and very small ovaries. BMR, glucose tolerance test, insulin tolerance test and 17 KS concentration in urine were normal. A low concentration of FSH excretion in urine was noted. X-ray of sella turcica was normal. Since 1956 continuing change of the face with coarser features and bigger hands and feet. Still amenorrhoea.



Fig 1 Case 1 in childhood.

I 1966 reexamined at hospital because of suspected acromegaly (Fig. 2). Marked cheekbones and superciliary arches. Hairy face ith deep furrows, greasy and perspiring skin. No cutis vertices gyrata. Big heavy hands and feet with deeply furrowed skin. Heavy legs. Increased growth of hair on arms and legs but normal pubic hair. Undeveloped mammae. External genitalia including clitoris normal. Still amenorrhoea.

At examination normal routine blood and urine analyses. Sodium in plasma normal. Glucose tolerance test, insulin tolerance test, cortisol in plasma during 24 hours, 17 KS and 17 KGS excretion in urine, PBI, triiodothyronine, BMR were normal. FSH excretion in urine less than 10 mouse-units and oestrogen excretion in urine 4.6 μg, 24 h, the two latter being pathologically low values. X-ray of chest and sella turcica normal. X-ray f the extremities did not show any packyperiostosis. The fields of vision normal. Analysis of the chromosomes in 19 lymphocytes showed normal female caryotype.

Since the patient told us that her mother had had a similar appearance and had been dead for many years, we tried to trace old hospital records and photographs (Fig. 3) and found the following information.

Case

In 1925 the patient, born in 1900, came to the hospital because of irregular menses. Menstruation did not begin until she was 20 years old. Menses occurred only during the summer She had an acromegal appearance ith furunculosis. The uterus was small and adnexa not palpable. BMR and X ray of sella turcica were normal.

In 1932 repeated examinations because of suspected acromegaly Since this year regular and normal menstruation. Examination showed big hands. Heavy and prominent chin and cheeks, marked superciliary arches. Normal field of vision. Investigation showed BMR −16, normal glucose tolerance test and normal X-ray of sella turcica. In 1937 pregnant. Sectio cesarea because of toxaemia of pregnancy Died from postoperative haemorrhage. The necropsy otherwise normal, showed a macroscopically enlarged pituitary N adenoma was obvious. At microscopy no eosinophilic adenoma was seen.

DISCUSSION

Regarding the diagnosis these two patients obviously belong to the group pachydermoperiostosis in accordance with the definition of the disease (21 25 26) They are closest to the type forme fruste in which the main changes shown are pachydermia in face hands and feet. The most important differential diagnosis is from hypertrophic osteoarthropathy which is a more common



Fig 2 Case 1 at the age of thirty

disease. According to the definition the latter disease is combined with painful joint symptoms and secondary to chronic lung disease. In neither of our cases was there arthropathy or lung disease. Normal lungs were shown in the necropsy and in the other case the chest X-ray was normal

The observation in the records of the birth of *the daughter*, case 1 that the child had an acromegal appearance is unique. As a rule the patients show the first symptoms just before puberty and development of the disease is over when they reach middle age.

The inheritance factor in this disease has been known from the first published cases (8) Vague (26) gives the proportion 40% and Angel (1) 25% Vogl and Goldfischer (28) published a list of known cases up to that date taken from 22 earlier papers. Three more cases have been added to that list (9 16 22) For all earlier reported cases there has been an identity of disease between brothers, brother–sister father–son and father–daughter But in no earlier case has the disease been seen in mother as well as daughter. The hereditary mechanism is not known. The only cytogenetic examination, as far as the author knows, has been that by Tzoneva (22), who found in father and son 47 chromosomes at analysis of white blood cells. Close examination of the caryotypes indicated that both probably had XXY trisomy while the mother had 46 chromosomes with normal female caryotype. An analysis of the lymphocytes of case 1 showed throughout female caryotype. Pachydermoperiostosis was earlier thought to be an exclusively male disease. Today however there is a list of ten female cases (3 4 6, 10 13 14 17 18, 28, 29), the diagnosis of which, however is proved only in six cases which were actually examined by the authors of the articles.

Tornblom et al. (23) pointed out the disturbances of sodium balance in pachydermoperiostosis, and therefore we examined the plasma sodium which, however was found to be at normal concentration.

The similarities between acromegaly and pachydermoperiostosis have resulted in confusion between these two diseases as well as the suggestion that pachydermoperiostosis may have an endocrine etiology Grönberg (11) published ten cases of cutis verticis gyrata and acromegal features, thus a clinical picture resembling pachydermoperiostosis, but in no case was he able to prove



Fig 3. Case 2. Mother of case 1

tumour of the pituitary Fried (7) reported two cases of osteoarthropathy and bronchial cancer At necropsy both cases had hyperplasia of the eosinophilic cells of the anterior lobe of the pituitary Williams and Celestin (30) between 1959 and 1961 found three published cases of bronchial carcinoma combined with acromegaly Recently Steiner et al. (19) published a case of bronchial carcinoma and osteoarthropathy Before operation the patient showed an increased concentration of the plasma HGH. After resection of an adenocarcinoma of the left upper lung lobe, the plasma HGH decreased to normal level.

Furthermore Gaughey (9) reported a 25-year old man with symptoms of pachydermoperiostosis during ten years and eunuch body proportions. Biopsy of the testis showed atrophy and depression of the spermatogenesis. Gaughey mentioned in his paper that experimental hypogonadism in rats is a precursor of pituitary overactivity He postulates that the release of the anterior pituitary gland from the inhibition of blood androgens and oestrogens may in turn lead to an increase of

growth hormone, i.e. pituitary function other than gonadotrophic function may be increased when androgenic function fails.

But for the difference in sex, there are great similarities between this last case and the present two It is probable that case 2, in spite of pregnancy *suffered from some degree of gonadal* hypofunction since she did not begin menstruation until she was 20 and even then the menses were fairly scanty She became pregnant at the age of 37 years and died at delivery Necropsy showed a microscopically normal pituitary and no adenoma was found. The daughter now 30 years old, still suffers from amenorrhoea. Gynaecological examination showed a small uterus and atrophy of the ovaries. In thorough examination of the endocrine functions the only pathological finding was a low concentration of the oestrogens and FSH excretion in the urine. We were able to analyse the concentration of the growth hormone, STH and this was normal. Thus in this case there was no evidence of compensatory overactivity of the pituitary cells producing STH as was postulated by Gaughey (9) *It is at the moment impossible to decide whether* pachydermoperiostosis is a consequence of hypogonadism or whether both are results of genetic damage It seems important to subject every case of pachydermoperiostosis to a thorough endocrine examination.

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BODY COMPOSITION IN OBESITY[1]

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Abstract. Thirty-nine obese patients and 19 clinical variables have been studied, and about 300 correlations between them were analyzed. The following observations were made:

1. The overweight predicted only on the basis of height is about the same as that calculated from height and skeletal measurements.

2. Calculations of the body-fat and lean body mass based on ^{40}K determinations show that not only the fat content but also the LBM seem to be increased in obesity especially in men. In agreement with this a significantly higher total body potassium and LBM was found in the men but not in the women in comparison with normal subjects. The difference in the amount of fat content between the obese and the normal subjects was statistically significant in both sexes. The highest amount of fat found was 90 kg, the lowest, in a control subject, 7 kg.

3. An equation is given which allows the body-fat to be calculated without ^{40}K determinations. Only the thickness of the subscapular and paraumbilical skinfold, the weight, and the height are needed.

4. In contrast to those of normal weight, obese women—possibly owing to the high caloric intake—have as high serum iron as men but despite this lower haemoglobin level than the men.

5. The blood volume rises with weight, as in subjects of normal weight.

6. In the women the serum concentration of iron, folic acid and protein fall with rising weight. The serum concentration of ascorbic acid is low especially in men.

7. The obese patients have normal serum cholesterol level, but the fasting blood sugar, serum triglycerides and blood pressure are raised.

8. The oxygen consumption is significantly correlated to the body cell mass, which confirms earlier observations.

Since it is said that weight reduction reduces the increased morbidity and mortality in overweight patients (26) an attempt was made to study the body composition. Prognosis concerning complications and therapy might be different in patients with different body compositions.

The determination of ^{40}K in a whole body counter offers a convenient way of estimating the body composition (10, 15, 16, 8).

In the present study the relations between lean body mass as calculated from ^{40}K to other parameters of body composition and of nutrition were analyzed. Relevant previous studies have been reviewed earlier (21) and the change during total fasting in the relations found here will be described subsequently.

MATERIAL AND METHODS

Controls

Ten women and nine men, apparently healthy among the hospital staff served as controls. The age of the men varied between 30 and 57 years (average 38.2), and of the women between 28 and 57 years (average 43.2).

Patients and complicating disease

Thirty-nine patients were studied, hospitalized only for treatment of obesity by total fasting, 15 men and 24 women. All variables could not be studied in all patients for various reasons. One was unsatisfactory determination of ^{47}Ca and ^{40}K in some initial patients, another the loss of several random laboratory samples. The values reported are unselected. The missing values are unlikely to bias the statistics reported. The age of the men varied between 23 and 64 years (average 42.7) and that of the women between 20 and 73 years (average 46.0).

An elevated blood pressure was discovered in three men and two women, and elevated fasting blood sugar values (> 120 mg%) were discovered in six men and eight women. The ECG showed signs of coronary insufficiency over the left ventricle in two of the men with hypertension. No other signs of disease were found in the history or physical examination.

[1] Preliminary reports of this paper have been published in Transactions of the Swedish Physicians National Conference 1964 (p. 89) and 1965 (p. 95).

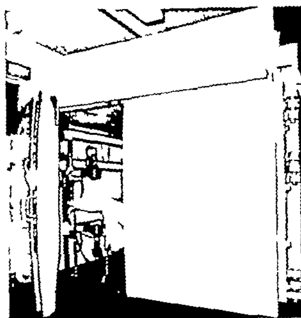


Fig. 1. The whole body counter.

Weight and potassium

The patients were weighed on standard scales. The height was measured without shoes and to within 0.5 cm.

^{40}K, which comprises a large part of the body's natural radioactivity, was determined in a whole body counter. The patient was placed in the horizontal position on a trolley and the radioactivity was measured with two scanning sodium iodide crystals, one above and one below the patient (Fig. 1). A detailed description of method and apparatus has been given (20).



Fig. 2. The whole body counter with the phantom in position.

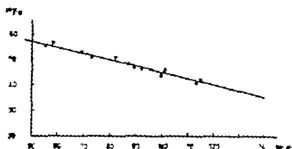


Fig. 3. The efficiency (y) at various body weights (x) of the whole body counter in the energy channel 1.3–1.5 MeV ($y = -0.25X + 69.5$).

Calibration

A phantom consisting of plastic bottles (Fig. 2) filled with KCl-solution, 141 g potassium in 85 l distilled water was used. To correct for the absorption of gamma-rays in the body-fat 13 healthy male volunteers received 1 μC ^{59}Fe, the energy of which (1.20 MeV) lies near that of ^{40}K (1.46 MeV). In this manner the efficiencies at various body weights in the whole body counter could be estimated (Fig. 3), and the ^{40}K-values obtained in obese patients could be corrected. The zero-point was set at 85 kg, since the phantom contained 85 l water. The equation of regression was calculated with a computer.

The thickness of subcutaneous skinfolds was measured subscapularly (dorsal fold), in the axillary line at mamillary level (thoracic fold) and paraumbilically in the medioclavicular line (abdominal fold), all on the right side of the body. The technique and the apparatus are the same as described by Hallström (18) and Lindegård (23).

Blood and nutrition

The blood volume was determined by the carbon monoxide method according to Sjöstrand (37, 38). The following analyses were performed in the Haematology Laboratory: the haemoglobin concentration by the cyanmethaemoglobin method, the reticulocyte count after supravital staining and serum iron with ofenantrolin. The serum folic acid determinations were made with Lactobacillus casei (31). The C-vitamin content of serum was determined according to Lowry et al. (25) and Roe and Kuether (33). Serum vitamin A determinations have been described before (30).

The following analyses were performed in the Central Chemistry Laboratory (Head: B. Swedin) by standard methods: serum proteins, paper electrophoresis, protein-bound iodine in serum, serum cholesterol and fasting blood sugar. The serum triglycerides were determined according to and by L. A. Carlson (8).

The basal metabolic rate was estimated at the Central Physiologic Laboratory and 17-keto and ketogenic steroids in urine at the Hormone Laboratory (Head: E. Diczfalusy). The blood pressure was recorded in the recumbent position.

The statistical analyses were made with an IBM 7090

Table I. *Mean weight and height in the present series*

Sex		Weight, kg			Height, cm		
		Mean	S.D.	S.E.	Mean	S.D.	S.E.
♂	15	117.1	26.9	6.7	178.5	8.0	2.0
♀	24	97.8	19.0	3.6	163.8	6.1	1.2

computer (41). All mean values and direct linear correlations are calculated. Significant correlations are selected by the computer (41).

Definitions

The statistical symbols used are the following:

- r – coefficient of correlation
- *b* – regression coefficient
- Sb – standard deviation of regression coefficient
- n – number of samples
- * – significant correlation at the 5% level
- ** – significant correlation at the 1% level
- *** – significant correlation at the 0.1% level

According to the original definition the fat-free body weight (FFW) denotes the part of the body in which the energetic exchange is assumed to take place and which was regarded as containing a *relatively homogeneous* mass of cells. It necessarily contains a small part of the metabolically little active tissue, i.e., the supportive tissue (27).

The lean body mass (LBM) initially denoted a part of the body with constant specific gravity and consists of a heterogeneous tissue mass. It comprises the whole body minus neutral fat and contains 2–10% essential lipids. The LBM is thus not completely identical with the FFW (27).

RESULTS AND DISCUSSION

Weight and body composition

The results in the controls (Tables II and III) are in agreement with the earlier literature (16, 18). The mean weight in the obese men was 117.1 kg and in women 97.8 kg (Table I). The mean overweight, evaluated on the basis of the maximum normal weight calculated according to von Döbeln's formula (1), was 36.3 kg in the men and 31.5 kg in the women. The mean percentage overweight was 47% in the former and 49% in the latter. If a comparison is made between the maximum normal weight according to von Döbeln's formula (12) (which takes into account both the height and the so-called sturdiness factor measured in this case as the wrist width) on the one hand, and the so-called centimetre weight (height in cm minus 100 = ideal weight in kg) on the other, no essential differences are found (Fig. 4). It is only in extremely sturdy subjects that some overestimation of the overweight occurs with the centimetre weight as basis. However for clinical practical purposes the centimetre weight can be used in most cases.

The mean height was 178.5 cm in the men

Table II. *Total body potassium in controls and obese subjects*

Reference	Subjects	Sex	n	Age (y.)	Weight (kg)	Mean (g K)	S.D.	S.E.
Present results	Obese	♂	9	42.2	113.9	191.7	32.2	10.7
		♀	15	42.4	97.5	123.6	20.7	5.3
Present results	Controls	♂	9	38.2	75.6	158.0	15.7	5.2
		♀	10	43.2	60.2	110.2	15.0	4.8
10	Obese	♂		35.6	100.9	168.1		
		♀		36.8	81.8	109.8		
31	Obese	♂		42.5	153.6	195.8		

Table III. *Body composition calculated from total body potassium*

Subjects	Sex	n	Age (y.)	Weight, kg Mean	S.D.	S.E.	LBM, kg Mean	S.D.	S.E.	Fat, kg Mean	S.D.	S.E.	Fat, % Mean	S.D.	S.E.	BCM, kg Mean	S.D.	S.E.
Obese	♂	9	42.2	113.9	19.7	6.6	72.0	12.1	4.0	41.9	13.6	4.5	36.3	8.3	2.8	40.8	6.8	2.3
	♀	15	42.4	97.5	22.0	5.6	46.4	7.8	2.0	52.4	17.8	4.6	52.1	8.1	2.1	26.3	4.4	1.1
Controls	♂	9	38.2	75.6	6.7	2.2	59.4	5.9	2.0	16.2	7.1	2.4	21	8.3	2.8	33.7	3.4	1.1
	♀	10	43.2	60.2	7.0	2.2	41.4	5.6	1.8	18.8	6.2	2.0	30.8	8.9	2.8	23.5	3.2	1.0

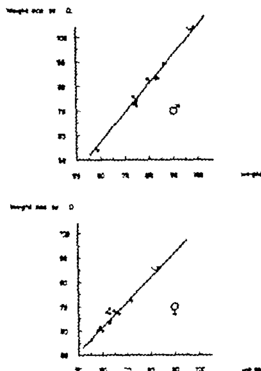


Fig 4 Relation between normal weight index according to von Döbeln formula (12) and centimetre weight" (see text). (L_1 $Y = 1.191$ $X - 14.1$ L_2 $Y = 1.016$ $X + 0.33$)

and 163 8 cm in the women. A significant correlation between weight and height was present in both sexes (*Table I*).

The total body potassium calculated from ^{40}K measurements was determined in 24 of the 39 patients and was 191 7 g in the men, which corresponds to 1.68 g/kg body weight. The corresponding values in the women were 123 6 g and 1.27 g/kg body weight. These values are in good agreement with those in the earlier literature (10 24, 28) and were also confirmed in simultaneous studies (Table II). The difference between the total potassium in men and women is significant, and so are the values calculated as g/kg body weight.

Attempts have been made to use the body's potassium content to calculate the LBM since this correlation was shown in animal experiments to be relatively good. Chemical analyses of the potassium content in man also gave fairly constant values, with a mean of 68 1 (range 66.5–72.8) mEq/kg LBM (14). In addition, LBM calculated from total body water determinations agree well with those obtained from ^{40}K (15) Therefore the potassium content of the LBM in healthy subjects is considered to be relatively constant (15) Recent analyses of the electrolyte content of muscle, which actually contains the greater part of the body's potassium by neutron activation further show the potassium content to be comparatively constant in healthy subjects (3)

Instead of using weight and body surface area or lean body mass and fat free weight, which are chemically and energetically heterogenous and contain both active cells and supportive tissue as reference unit in evaluation of energy exchange and basal metabolism, it has been advocated to use the fictive expression body cell mass (BCM) which is chemically homogenous and comprises the energetically active part of the body (27). This can be calculated from the total body potassium according to the formula. total potassium in mEq × 8.33 = BCM in g, where the factor 8.33 is obtained both from the ratio of potassium to nitrogen and to wet lean tissue (27) In order to permit comparisons both LBM and BCM were calculated in the present series.

Our patients had an increased LBM of 72 kg for men and of 46 kg for women (Table III) as compared to that in control series reported in the literature (16) 55 kg for men and 35 for women around 40 years of age. These values are in good agreement with our own control values (Table III) The increased LBM is also illustrated by the significant correlation between ^{40}K and weight, since LBM is calculated as a function of body potassium. In the men there is also a significant correlation between ^{40}K and height (Table IV). The patients mean BCM values are also listed in Table III Like LBM these

Table IV *Correlation between weight height and total body potassium*

Sex			b	Sb	Stat signif.
Weight and total body potassium					
♂	9	0.703	1.239	4 706 10^{-}	
♀	15	0.782	7.300 10^{-}	1 612 10^{-}	
Height and total body potassium					
♂	9	0 670	2.491	1.044	
♀	15	−0 107	−7 139 10^{-}	6 135 10^{-4}	—

Table V *Thickness of skinfolds (mm) at different sites*

				Subscapular			Axillary			Paraumbilical		
Reference	Subjects	Sex		Mean	S D	S.E.	Mean	S D	S.E.	Mean	S.D	S.E.
Present results	Obese	♂	8	36.0	12.6	4.4	26.7	6 9	2.4	31.7	12.0	4.3
		♀	16	32.7	9 7	2.4	22.9	5.5	1 4	38.0	11.3	2.8
Present results	Controls	♂	8	13.9	4.4	1.6	17.3	5 7	2.0	29.3	6.3	2.2
		♀	9	9.3	0.3	0.1	10.5	4.5	1.5	15 0	8.2	2.7
16		♂		8.0	3 6	—	6.7	4.3	—	8.3	4 9	—

Table VI. *Correlation between body weight and skinfold thickness at different sites*

Sex	Sites			b	Sb	Stat. signif.
♂	Subsc.	8	0 945	3.363 $10^{-\text{[illegible]}}$	4 767 $10^{-\text{[illegible]}}$	
	Axill.	8	0 644	1.261 $10^{-\text{[illegible]}}$	6.116 $10^{-\text{[illegible]}}$	
	Paraumbil.	8	0.356	1.215 $10^{-\text{[illegible]}}$	1.302 $10^{-\text{[illegible]}}$	
♀	Subsc.	16	0.669	3.080 $10^{-\text{[illegible]}}$	9 136 $10^{-\text{[illegible]}}$	
	Axill.	15*	0.052	1.339 $10^{-\text{[illegible]}}$	7.075 $10^{-\text{[illegible]}}$	
	Paraumbil.	16	0 894	4.794 $10^{-\text{[illegible]}}$	6 406 $10^{-\text{[illegible]}}$	

One measurement omitted accidentally

values are above those in subjects of normal weight.

The high BCM in obesity is also illustrated by studies using Sheldon's classification. Endo- and mesomorphs with high BCMs are over-represent ed in series of obese subjects, and the endomorphs in particular have a tendency to accumulate more fat than the meso- and ectomorphs (22, 29 35)

Table III also shows the calculated fat mass. As could be expected (17) the percentage of fat was greater in women than in men. The differ ence is statistically significant. A considerable variation in the amount of fat in the body was found. The leanest normal person had 7 kg, the fattest patient 90 kg. In the future, studies of the implications of the amounts and percentages of fat on blood sugar and lipids, for instance, will be of interest. Obese patients of both sexes had a distinctly increased percentage of fat as compared to subjects of normal weight, in whom, according both to earlier and the present studies, at 40 years of age it comprises 15–20% of the total body weight in men and 20–30% in women (1 11) (Table III).

The results of skinfold measurements are seen in Table V As a comparison values from subjects of normal weight are included, and also Hellström's values of 70-year-old men (18), al though we recognize the difference in ge. A significant positive correlation exists between height and axillary and subscapular skinfolds in the men and between height and paraumbilical skinfolds in women. In the men a highly significant correlation is present between weight and subscapular skinfold thickness and an almost significant correlation between weight and axillary

Table VII. *Correlation between subcutaneous skinfold thickness at different sites*

Sex	Sites			b	Sb	Stat. signif.
♂	Subscap.-axill	8	0.742	4 081 $10^{-\text{[illegible]}}$	1 505 $10^{-\text{[illegible]}}$	
	Subscap -paraumbil.	8	0.335	3.211 $10^{-\text{[illegible]}}$	3.687 $10^{-\text{[illegible]}}$	—
	Axill.-paraumbil.	8	0.297	5 171 10^{-}	6 795 $10^{-\text{[illegible]}}$	—
♀	Subscap -axill.	15	−0 098	−5.371 $10^{-\text{[illegible]}}$	1 516 $10^{-\text{[illegible]}}$	—
	Subscap -paraumbil.	16	0 658	7.660 $10^{-\text{[illegible]}}$	2 345 $10^{-\text{[illegible]}}$	
	Axill.-paraumbil.	15	0 356	7 448 $10^{-\text{[illegible]}}$	5 421 $10^{-\text{[illegible]}}$	—

Table VIII. *Fat mass according to total body potassium and skinfold thickness*[a]

Sex	n	Method	Mean	S D	S.E.
♂	8	^{40}K	40.5	13.7	4.8
		Skinfold	38.6	12.9	4.5
	12	^{40}K	53.5	19.9	5.7
		Skinfold	53.1	20.5	5.9

The fat mass in men was calculated

$$= \frac{\text{dorsal} + \text{abdominal skinfold}}{2} \times \text{body surface area} \times 0.9$$

and in women

$$= \frac{\text{dorsal} + \text{abdominal skinfold}}{2} \times \text{body surface area} \times 0.9 \times 1.56.$$

skinfold. In the women the correlation is significant between weight and both subscapular and paraumbilical skinfold thickness (Table VI). The fact that both the skinfold thickness and ^{40}K are significantly related to the patients' weight also confirms the observation that the increased weight depends both on an increased amount of fat and an increased amount of LBM.

The relation between the measurements at different sites is shown in Table VII. A significant correlation is present in men between the thickness of the axillary and subscapular skinfolds and in women between the paraumbilical and subscapular skinfolds. It thus seems as if the subscapular skinfold thickness is the best expression of the degree of obesity of these three. This is also in agreement with other observations, on the basis of which it was suggested that the subscapular skinfold, which has the most well-defined localization, is one of the best criteria of obesity (36).

It was of interest to study the relation between the fat mass obtained by multiplying the body surface by the thickness of subcutaneous skinfold to that calculated from ^{40}K measurements. The mean half of two skinfolds (subscapular and paraumbilical) was used. The specific gravity of fat was assumed to be 0.9

(Fat in kg in men

$$= \frac{0.5\ (\text{dorsal} + \text{abdominal skinfold})}{2} \times \text{body surface area} \times 0.9\ )$$

Good agreement was found in men. In women a mean ratio of 1.56 between fat calculated from ^{40}K and the values calculated from skinfold thickness was found (Table VIII). This can be explained by body configuration, fat distribution, etc. However, there was a significant correlation in women as well between the results of the two methods for estimation of the fat mass. A tentative calculation of the total body fat can thus be made in obese patients without access to a whole body counter or any other complicated apparatus for determination of body composition.

Haematology

The mean value for the blood volume was 4.57 l in women (Table IX) and 5.78 l in men. A significant correlation is present at the 1% level between weight and blood volume. In men the same significance was present between height and blood volume, and between blood volume and ^{40}K. The data show that these previously known correlations are valid also in obesity (39). These conditions are studied in detail in a separate paper.

The mean values for the haemoglobin, serum iron and folic acid concentrations in serum are listed in Table IX. All the individual values were well within the normal limits. The difference between haemoglobin in men and women is sig-

Table IX. *Haematological mean values*

Sex	n	Hb (g/100 ml) Mean	S.D.	S.E.	n	Serum Fe (mg/100 ml) Mean	S.D.	S.E.	n	Folic acid (mμg/ml) Mean	S.D.	S.E.	n	Blood volume (l) Mean	S.D.	S.E.
	1[illegible]	14.6	±1.3	0.4	13	0.130	±0.054	0.015	9	6.75	±[illegible].44	0.73	8	5.78	1.[illegible]6	0.48
	23	13.1	±1.[illegible]	0.3	23	0.109	±0.030	0.006	20	6.8	±2.9	0.6	13	4.57	0.63	0.19

Table X. *Nutritional mean values*

Sex		Serum protein (g/100 ml) Mean	S.D.	S.E.		Vitamin C (mg/100 ml) Mean	S.D.	S.E.		Vitamin A (IU) Mean	S.D.	S.E.
♂	15	7.9	±0.7	0.2	7	0.53	±0.34	0.13	6	146	±36	15
♀	28	7.7	±0.6	0.1	7	0.63	±0.26	0.10	4	159	±52	26

Table XI. *Correlation between body weight and certain nutritional factors*

Sex				*b*	Sb	Stat. signif.
♂	Serum F	13	0.026	5.146 10⁻⁴	5.931 10⁻⁴	
	Folic acid	9	−0.305	−7.221 10⁻	8.538 10	
	Serum protein	15	−0.154	−3.905 10⁻⁴	6.958 10⁻	
♀	Serum Fe	28	−0.489	−7.761 10⁻⁴	2.713 10⁻⁴	
	Folic acid	20	−0.526	−7.732 10⁻⁴	2.947 10⁻⁴	
	Serum protein	28	−0.606	−1.917 10	4.941 10⁻⁴	

nificant, whereas this does not apply to serum iron and folic acid. Usually healthy women in fertile age groups have lower serum irons than men. One possible explanation may be the over consumption of nutrients which supply sufficient iron to cover the loss even in menstruating women.

Nutrition

Table X shows the mean values of other nutritional parameters. As in the case of serum iron and folic acid an unexplained but significant negative correlation is found in women between serum protein and weight (Table XI). This correlation is not significant in the men, but here also the coefficient of correlation is negative for serum protein and folic acid.

The mean vitamin C value in men is close to the lower limit, 0.5 mg/100 ml. We did in fact find values below this in four men but without the existence of deficiency symptoms. All the four men were unmarried and food habits may explain some of the findings. Obesity thus does not seem to imply high serum levels of vitamin C, folic acid or protein.

In agreement with most earlier studies of serum lipids in the obese (2, 7) we noted normal serum cholesterol values (6, 9) in both men and women, whereas the mean serum triglycerides were raised in the men and normal in the women (6, 9, 34) (Table XII). The difference between men and women with respect to serum cholesterol and triglycerides is not significant even if a tendency to higher values is present in the men in both cases.

The mean values for fasting blood sugar are also given in Table XII. The mean value in men is slightly higher than the upper normal limit for normal individuals, 120 mg/100 ml with the method used (13, 19). In women the mean was at the upper normal limit. The difference between men and women is not, however, significant. It

Table XII. *Mean values of blood lipids and fasting blood sugar*

Sex		Cholesterol (mg/100 ml) Mean	S.D.	S.E.		Triglycerides (mmol/l) Mean	S.D.	S.E.		Blood sugar (mg/100 ml) Mean	S.D.	S.E.		Blood pressure, systolic (mm Hg) Mean	S.D.	S.E.		Blood pressure, diastolic (mm Hg) Mean	S.D.	S.E.
♂	11	297.7	±35.7	10.8	7	2.86	±2.03	0.77	15	130.5	±44.2	11.4	14	145.4	±31.7	8.5	14	93.2	±14.9	4.0
♀	19	256.1	±38	8.8	13	1.46	±0.89	0.25	26	119.0	±30.9	6.1	28	141.6	±28.9	5.5	28	89.5	±17.9	3.4

Table XIII. *Correlation between fasting blood sugar and serum triglycerides*

Sex	n	r	b	Sb	Stat. signif.
♂	7	0 811	2.246×10^{-4}	7.236	
♀	12	0.467	7 735	4 627	—

Table XIV *Oxygen consumption and correlation to total body potassium*

Sex		O_2 consumption (l/min) Mean	S.D	S.E.		Stat. signif
♂	6	0.296	0 072	0.029	0 73	
♀	9	0.229	0.032	0 011	0.94	

has previously been shown that obesity is often associated with latent or manifest diabetes mellitus (4) and this has been confirmed in recent studies (2) In six men and eight women the fasting blood sugar was moderately elevated two men and one woman subsequently required tablets and dietary treatment.

A correlation has been demonstrated to exist between a high serum triglyceride level and obesity and this applies to a still higher degree to high serum triglycerides and diabetes mellitus (34) In the present series a significant correlation is present at the 5% level between serum triglycerides and fasting blood sugar in the men but not in the women (Table XIII)

Other data

A definite correlation exists between obesity and high blood pressure which is associated with fundus hypertonicus changes (2) The mean values for the blood pressure in the present series are given in Table XII and agree with those found earlier (2, 26) A significant correlation at the 5% level is present between weight and diastolic pressure in the women. The blood pressure was registered with an ordinary cuff and no correction was made for the circumference of the arm. Too high values could therefore be expected. These details are being further studied and will be published separately

No raised excretion of 17-keto or ketogenic steroids was recorded in any of the patients.

The protein-bound iodide (PBI) was 6.7 ± S.D 1.3 μg/100 ml in the men and in the women 5 9 ± S.D 1 6 μg/100 ml.

The mean value of the BMR was +14 ± S.E. 7% in the men, and −6 ± S.E. 4% in the women. The difference between the sexes is significant at the 5% level. The mean values of the oxygen consumption was 0.296 l/min in the men and 0.229 l/min in the women. The difference is statistically significant. The correlation between total body potassium and oxygen consumption is statistically significant in both men and women (Table XIV).

The oxygen consumption increases with rising body weight but is lower per kg weight in obese subjects than in those of normal weight. The increased total oxygen consumption has been shown to be well correlated to the increased cell mass (40) This may explain why men having higher BCMs, have higher BMRs than women.

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EFFECT OF STARVATION ON BODY COMPOSITION IN OBESITY

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Abstract. 1. Thirty-nine patients with comparatively pronounced obesity underwent total starvation treatment for periods ranging from two to nine weeks, on an average four weeks. Relatively few subjective discomforts were experienced. Two patients discontinued the treatment, both after about ten days. Eight patients had eaten food without permission on some occasions, but in general they subsequently adhered to the total starvation diet.

2. No serious complications were observed. In one third of the patients hypokalaemia developed, but the intracellular potassium concentration also decreased, so that only a few of these patients had ST T-changes in the ECG. There were no disturbances in cardiac rhythm. Treatment with potassium rapidly normalized the serum potassium. In one-third of the patients the bicarbonate value was below 18 and bicarbonate was administered. No disturbances in blood pH were observed. As a rule the uric acid concentration increased, but decreased spontaneously after 2–3 weeks. No attacks of gout occurred.

3. Mean loss in weight was 12% of the initial weight. The most rapid decrease was observed during the first week. The men lost 0.39 kg per day and the women 0.43 kg.

4. The mean loss in ^{40}K was 30 g for women and 25 g for men. Only in the women was the decrease in ^{40}K statistically significant. In previous investigations the regression coefficient was calculated for the fat mass measured by means of the thickness of the skinfold in relation to that found by means of ^{40}K. This equation was used for calculating the lean body mass after starvation. The mean potassium content was 58.1 mEq/kg of the lean body mass after starvation, compared with an expected 68.1 mEq/kg in non-starving persons. This intracellular potassium depletion explains part of the potassium loss during starvation. Since the mean serum potassium diminished from 4.1 to 3.6 mEq/l the relation between intra- and extra-cellular potassium was relatively constant.

5. In men with obesity starvation causes moderate loss in lean body mass and a substantial loss in fat. In women there is also largely a loss of fat, but apparently more lean body mass is lost than in the men. Total haemoglobin was diminished in women, which is an expression of the loss in cellular mass during starvation in women.

6. The concentrations of ascorbic acid, sugar, serum iron and serum cholesterol decreased in the women, and the concentration of folic acid and triglycerides in serum in the men. Systolic blood pressure fell 0.5–0.6 mm Hg per day.

7. Fasting blood sugar and serum triglycerides were normalized during fasting in the men.

Since obesity is a common condition associated with increased morbidity and mortality, especially in diabetes mellitus and cardiovascular disease (14, 20) and, moreover, since these decrease on reduction of weight (20), different therapeutic methods have been developed in the course of time.

The studies described here were begun in 1964 (2, 16) following reports on attempts to treat obesity by means of total starvation (3, 5, 7, 9). These trials showed that even quite long periods of starvation are well tolerated, and that the risks involved are very slight. They mainly consisted in the occurrence of hypokalaemia in certain patients and an increase in uric acid, and hence the risk of gout attacks in persons prone to such attacks. The first trials (3) indicated that the rapid reduction in weight was primarily due to the breakdown of fat. Later, however, it was claimed that the loss in protein would exceed the loss in fat (21). The purpose of the investigations described here was, therefore, to further study the changes in body composition, haematology and nutrition which occur during starvation. Further trials made after these studies were begun have confirmed the original results regarding breakdown of fat (12, 13, ..., 24, 25).

MATERIAL AND METHODS

The material comprised 15 men and 24 women, with a total of 48 starvation periods. Ten men and five

Table I. *Changes in weight during starvation*

Sex	Period of treatment days (Mean)	Weight loss in kg (Mean)	Weight loss in % of initial weight (Mean)	Weight decrease per day[a] Mean	S.D.	S.E.
♂	_5.4	13.8	1_0	0.59	0.19	0.04
♀	20.5	12.1	1_8	0.43	0.08	0.01

[a] Average calculated on the assumption that decrease in weight was linear function of time. Actually at the beginning of the starvation period the weight decreased more, and at the end less than shown here (Fig. 1).

women each underwent two periods of starvation, and one man underwent three periods. The original composition of the material and the laboratory methods applied have been reported on separately (*18*).

Therapy

Most of the patients stayed in a special ward where there were no other patients. Thus they did not have any access to food in the ward. The patients received liquid ad libitum, water, mineral water, coffee, tea, and where vitamin studies were not made, multivitamin tablets. These were analyzed for folic acid activity but none was found. In case of need antacids, appetite reductants and psychopharmacological agents were given. The patients were treated as ambulants. They took walks, and individual patients trained on ergometer cycles and in gymnastic groups. They helped with the ordinary work at the ward.

Body composition and haematology

The patients were weighed every morning. The intake of liquid and the amount of urine were recorded per 24 hours to control that the intake was enough and that none was diuretic. ^{40}K was measured twice a week, and skinfolds once a week. For certain patients blood volume was determined before and towards the end of the starvation period, and in some others it was determined once again in the middle of the period. Serum iron was determined every other week. Folic acid, haemoglobin and reticulocytes were measured once a week; in a number of patients folic acid clearance was carried out towards the end of the starvation period.

Nutrition

Serum protein, vitamin C, vitamin A, as well as cholesterol, triglycerides, fasting blood sugar, serum electrolytes, blood gas analyses and uric acid were checked once a week.

Other data

Legal' test was made semiquantitatively every morning. Blood pressure was determined both in recumbent and standing position three times weekly. ECG was recorded once a week. B.M.R. and protein-bound iodine were checked once a week in a number of patients. Computers were used for part of the statistical analysis. The correlation and regression coefficients for each parameter upon time were calculated with an automatic data processing program, which has been previously described (26).

RESULTS AND DISCUSSION

Subjective reactions

The patients' feeling of hunger disappeared after 3–7 days, i.e. at about the same time as the ketosis occurred. Some of the patients denied that they had felt any feeling of hunger. During the starvation period many patients complained of the monotonous taste of both coffee and tea. Complaints were also made about having a bad taste in the mouth and bad breath, which was *due to the ketosis. After 2–3* weeks *there* was a certain degree of tiredness and psychic irritability and some of the patients found it difficult to concentrate when reading. These symptoms, however, did not cause any special difficulties. All the patients, with the exception of two, completed the starvation cure. The exceptions consisted of a young woman who found the stay in the hospital boring, and a woman in early middle age who considered that the reduction in weight was not sufficiently rapid. Both discontinued treatment after about ten days (the time *planned* was between 4 and 5 weeks).

As far as we were able to ascertain four men and four women had, on some occasion, eaten something during the starvation periods. The excretion of ketone bodies, which usually was high during the starvation period, thereby decreased rapidly. When questioned, the patients admitted that they *had eaten something,* but *afterwards* they did not "cheat" again. It was usually a piece of bread and butter but nothing involving

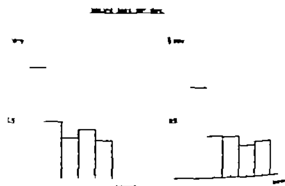


Fig. 1. Daily loss in weight for the men and the women.

Table II. *Body potassium content before and after starvation*

		g K/kg body weight			g total-K		
		Before	After		Before	After	
Sex		Mean S.E.	Mean S.E.	Significance	Mean S.E.	Mean S.E.	Significance
♂	9	1 70 ± 0.07	1.69 ± 0.11	$p > 0.05$	191 7 ± 10.7	167 1 ± 14 8	$p > 0.05$
♀	15	1.29 ± 0.05	1 13 ± 0.08	$p > 0.05$	123 6 ± 5.3	93.6 ± 5.7	$p < 0.001$

a large amount of calories. The usual reason was the monotony of the liquid diet and/or the need to chew

There was no defaecation after the first week, but a few of the patients felt congested during the period of starvation, and asked to be given a lavage this was done, but without any results.

Weight—lean and fat

The average starvation period was about four weeks, ranging from 9 to 61 days for men, and from 13 to 53 days for women. During the starvation cure the patients lost about 13 kg, which represents some 12% of the initial weight (Table I) The mean weight loss per day was about half a kg for men, approximately 150 g higher than for the women this difference is statistically significant (Table I). The greatest loss in weight occurred during the first week, when for men it amounted 0.95 kg/day and for women 0 78 kg/day It subsequently decreased, for both men and women, to a comparatively constant value (Fig. 1). The great initial loss in weight was probably due mainly to water (23).

In general, the amount of ^{40}K diminished during starvation, but the decrease in individual patients was found to be statistically significant only in two of the nine men and in six of the 15 women. Calculated on the basis of the regression coefficient of ^{40}K upon time, the mean potassium loss for the men and the women was 24 6 g and 30.0 g respectively· the value differs significantly from 0 in the women. The mean potassium content per kg body weight decreased for women (Table II), and in all the individual patients total potassium showed a tendency to decrease.

The lean body mass (LBM) and the body cell mass (BCM) cannot be calculated on the basis of the ^{40}K determination in starving subjects. After this study had been completed, Drenick et al. (8) suggested that tissue depletion of potassium would occur during starvation. This conclusion was based on changes in the K N quotient in urine. The same conclusion can be drawn from Table III which shows for some selected patients losses in potassium, according to ^{40}K, compared with the maximal potassium losses calculated on the basis of weight loss, on the assumption that the entire loss in weight is made up of cell mass. The loss in potassium based on ^{40}K is greater than the calculated maximal loss, which must be due to potassium tissue depletion. This may explain why it was originally claimed that greater losses in cell mass occurred after starvation than after a ketogenic, high fat but low-caloric diet (4). If, in spite of this, the LBM losses are calculated on the basis of the ^{40}K measurements, then the mean value for men would be 9.3 kg and for women 11.3 kg. With reservation for what has been stated above, a hypothetical body composition has also been calculated on the

Table III *Measured and estimated loss f potassium*

Sex	K-loss acc to ^{40}K determination	Maximal K-loss estimated from weight loss[a]
♂	40.2	21.0
♀	53 5	26 6
♀	59.0	27 4
♀	39 7	26 9
♀	33 6	4 8
	64 6	17.3
♀	56 9	26.9

On the assumption that the entire loss of weight consisted of cell mass, which can hardly be the case The table shows the selected cases here the loss of potassium estimated in this way was lower than that measured, which indicates that the potassium concentration in the remaining cell mass decreases

Table IV *Body composition before and after starvation estimated according to ⁴⁰K*[a]

Sex	n		Before (Mean)	After (Mean)
♂	9	Weight, kg	113.9	99.4
		LBM, kg[b]	72.0	6[illegible]7
		Fat, kg	41.9	36.7
		Fat, %	36.3	36.7
		BCM, kg[c]	40.8	35.6
[illegible]	15	Weight, kg	97.5	86.0
		LBM, kg	46.4	35.1
		Fat, kg	52.4	50.9
		Fat, %	52.1	57.5
		BCM, kg	26.3	19.9

[a] The given values are hypothetical—a tissue depletion of potassium occurs during starvation.
[b] LBM = lean body mass.
[c] BCM = body cell mass.

basis of the ⁴⁰K results after starvation, this is shown in Table IV. The LBM is found to be substantially decreased after starvation. We have also tried to calculate body composition after starvation, without making any assumptions in respect of intracellular potassium concentration, by means of the thickness of the subscapular skinfold (Table V). These calculations have been made with the aid of the previously reported (18) regression equation for the correlation between thickness of

Table V *Comparison between body composition after starvation on ⁴⁰K and that based on thickness of skinfold*[a]

Sex			Before (Mean)	After (Mean)	
[illegible]	7	Weight	110.5	97	
			⁴⁰K and skinfold	⁴⁰K	Skinfold
		LBM[b]	72	64[illegible]	7[illegible]
		Fat	37.8	32.6	25
		Fat, %	3[illegible]8	33.7	25
	6	Weight	90.1	78.7	
		LBM	44.6	33.1	40.8
		Fat	4[illegible]	45.5	38.0
		Fat, %	49.8	57.3	46.5

[a] ⁴⁰K indicates loss in LBM plus loss of potassium from the tissues, and skinfold the loss in fat.
Regression equation for skinfold—amount of fat given earlier (18).
[b] LBM = lean body mass.
In two women, ⁴⁰K measurements erroneous. If included, even greater reductions in intracellular ⁴⁰K-concentrations would have resulted.

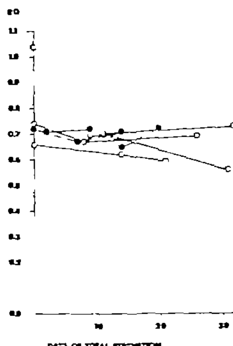


Fig —. Respiratory quotient during starvation.

skinfold and fat content. Such figures also make it possible to calculate potassium content, tentatively after starvation. This is 58.1 mEq/kg lean body mass, compared with a calculated figure of 68.1 mEq/kg for non-starving persons (11). Because the mean serum potassium decreased from 4.1 to 3.6 mEq/l, the potassium gradient over the cell wall was unchanged, i.e. 16.61 and 16.14 respectively. For this reason, probably no changes on the ECG of hypopotassaemia were found, apart from very small changes in a few patients. Lower serum potassium values than the lower normal limit of 3.2 mEq/l occurred in 17 out of 49 starvation periods.

The mean decrease in the skinfold was 0–0.4 mm/day. No significant difference between men and women or between various locations on the body were observed. The respiratory quotient (R.Q.) was determined in a few patients. Low values were obtained already before starvation, and thus there was only an insignificant decrease during starvation (Fig. —). The diminution of the skinfold and the small decrease and low R.Q. indicate that fat is consumed, and that the large losses of potassium are not only an expression of loss of cell mass but also of potassium tissue depletion.

Table VI *Haematological mean values before and after starvation*

Sex		Blood volume			Total Hb (g)			Hb (g/100 ml)			Reticulocytes (of all erythrocytes)			Serum iron (g 100 ml)			Folic acid (mμg ml)	
		Mean	S.E.		Mean	S.E.		Mean	S.E.		Mean	S.E.		Mean	S.E.		Mean	S.E.
♂ Before starvation	7	5.8[a]	0.6	7	818	37	12	14.6	0.4	12	7.6	0.9	18	130	15	15	6.8	0.7
After starvation	7	5.8	0.5	7	787	34	12	14.4	0.3	12	4.0	0.1	18	96	4	15	4.2	0
♀ Before starvation	9	4.4	0.1	9	622	31	23	13.1	0.3	20	6.2	0.8	27	109	6	19	6.8	0.6
After starvation	9	4.0	0.1	9	538	18	23	13.2	0.2	20	5.6	0.7	27	63	4	19	5.5	0

Unselected patients. All patients were not examined because of laboratory limitations.

Haematology

It has been previously described how erythropoiesis in mice regularly ceases during starvation. In order to study this effect in human beings, we have followed the haemoglobin concentration, blood volume, total haemoglobin, and reticulocytes. The blood volume was determined in seven men and seven women. There was a substantial reduction in the mean values of the women but not in those of the men. The total haemoglobin (Table VI) also showed statistically significant decrease in the mean values for the women, which amounted to 84 g. The corresponding figure for the men was 31 g, which was not statistically significant. This confirms the above mentioned finding of a more pronounced decrease in cell mass in the women. It has been suggested that the activity of housewives might be lower in the hospital than at home, but that of the men essentially the same. This could be a contributory explanation. No substantial decrease in the mean values for haemoglobin concentration was obtained. The mean value of the regression coefficient for the reticulocytes in relation to time was negative for both men and women, but did not differ significantly from 0 (Table VII).

Serum iron decreased substantially in the women, and folic acid in the men (Table VI). The decrease in folic acid was 0.07 μg/ml/day which is more than that described in Herbert's trials with folic acid deprivation during four months (15). The decrease in the women was 0.01 μg ml/day which is not statistically significant. In two men and four women the final concentration of folic acid in serum was less than 2.3 μg ml which was set as the lower limit. Folic acid clearance was studied in six patients (1 woman and 5 men) towards the end of starvation, but no statistically significant difference (as perhaps could be expected from the decrease in folic acid concentration in serum) was found in comparison with normal material (Table VIII).

Table VII. *Changes with time in certain haematological variables*

	Sex		Change/day Mean	S.E.	Degree of significance
Hb g	♂	12	−0.008	0.028	—
	♀	21	−0.007	0.015	—
Reticulocytes,	♂	12	−0.04	0.11	—
		20	−0.03	0.11	—
Serum iron, mg	♂	17	−0.0016	0.0009	—
		27	−0.0029	0.0002	
Folic acid, mμg/ml	♂	15	−0.07	0.03	—
	♀	19	−0.009	0.014	—

Probability that the regression coefficient, i.e. change per day differs significantly from 0.

Table VIII. *Folic acid clearance after 3–4 weeks starvation in obese persons in comparison with a normal material*

Reference		n	3 min Mean S.E.	15 min Mean S.E.	30 min Mean S.E.
Present study	After starvation	6	172±34	104±17	65±14
Ekman et al. (10)	Normal material		201±12	72±6	44±3

Nutrition

Vitamin C determinations were made, during the starvation period, for seven men and seven women. In two members of each group there was a statistically significant decrease in the values, and for the women the mean values also decreased significantly. The decrease amounted to 0.02 mg per 100 ml/day (Table IX). In all the patients the final values after starvation were below the given lower limit of 0.5 mg%. As is true for folic acid, results of this type, which apparently have not been previously published in respect of human beings, are of importance for calculating the ascorbic acid requirements in man. Opinions on these requirements vary by a factor of 10 (1). The vitamin A concentration in serum decreased significantly only in one woman, whereas there was no statistically significant decrease in the mean values either for the men or women (Table IX). The differences between the vitamins, the serum concentration of which remains constant for a long time without administration, such as vitamins A and B_{12}, and vitamins of which the concentration is rapidly reduced, such as folic acid and ascorbic acid, are of interest, among other things, for the investigation of nutritional disturbances in different diseases. In the clinical diagnosis of nutritional disturbance, the concentration of vitamins the concentration of which decreases rapidly should, of course, be investigated first.

Table IX. *Changes with time in certain nutritional values*

Nutrient	Sex		Change/day Mean	S.E.	Degree of significance*
Vitamin C	♂	7	0.021	0.006	
(mg/100 ml)		7	0.016	0.004	
Vitamin A	♂	6	−1.0	1.6	—
(I.U. ml)	♀	5	−1.7	1.6	—
Cholesterol	♂	14	−1.4	0.9	
(mg/100 ml)	♀	20	3.4	0.7	
Triglycerides	♂	11	−0.04	0.017	
(mmol/l)	♀	12	−0.04	0.03	
Fasting blood sugar	♂	19	1.4	0.6	
(mg/100 ml)	♀	30	−0.58	0.18	
Serum protein	♂	16	−0.009	0.007	—
(g)	♀	23	−0.017	0.006	

* Probability that the regression coefficient, i.e. change per day, differs significantly from 0.

It is a clinical experience that the concentration of protein and fat in serum is often decreased in nutritional disturbances. There was a statistically significant decrease in serum albumin in women but not in men (Table IX).

The oxygen consumption showed a slight decreasing tendency (Fig. 3). Decrease in protein-bound iodine (PBI) was not significant either in the men or women (Table X).

During starvation, changes take place in the

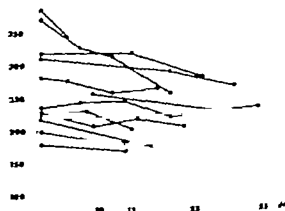


Fig. 3. Oxygen consumption during starvation.

Table X. Changes with time in other results

Variable	Sex	n	Change/day Mean	S.E.	Degree of significance
Systolic blood pressure	♂	18	−0.6	0.2	
(mm Hg)	♀	30	−0.5	0.1	
Diastolic blood pressure	♂	18	−0.3	0.2	—
(mm Hg)	♀	30	−0.2	0.1	—
PBI	♂	9	−0.11	0.05	—
(μg/100 ml)	♀	15	−0.04	0.06	—

fat metabolism, these have been studied in detail since the present studies were begun (19). Here, only changes occurring from week to week in the cholesterol and triglyceride concentrations in serum have been studied, while the more detailed studies will be published separately. The mean cholesterol concentration decreased significantly in women, but not in men, although the regression coefficient was negative. The greatest decrease measured in men was 148 mg% and in women 166 mg%. The mean triglyceride concentration decreased *significantly only in men.* This is an interesting finding in view of the importance of high triglyceride concentration for coronary disease in men (6).

There was a statistically significant decrease in blood sugar in both sexes. Despite the fact that low values, between 50–70 mg, occurred in a number of patients, no symptoms of hypoglycaemia occurred.

Prior to starvation the men's fasting blood sugar was raised, i.e. the men had a condition of mild diabetes. This appears to have been improved by starvation.

Other results

The systolic blood pressure decreased significantly in both men and women. The decrease per day was 0.6 and 0.5 mm respectively. Diastolic pressure decreased significantly in four men and six women, but the decrease in the mean values was not statistically significant (Table X). Hence starvation may cause a rapid and effective decrease in blood pressure. One contributory explanation could possibly be a decrease in the circumference of the arm. A few patients were troubled with orthostatism after about 2–3 weeks, which could easily be overcome by letting the patients rest a while before rising in the morning.

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Table I. *Distribution by age*

Age (y)	1950–52 (%)	1964–66 (%)
30–39	1.0	0.3
40–49	4.2	3.0
50–59	13.3	10.8
60–69	33.3	29.5
70–79	37.4	37.3
80–89	10.2	17.2
90–	0.6	1.9

Table II. *Distribution by diagnosis*

Diagnoses	1950–52 (%)	1964–66 (%)
Myocardial degeneration	61	53
Cardiac decompensation	47	52
Perpetual arrhythmia	45	20
Mitral disease	9	5
Aortic heart disease	3	2
Hypertensive heart disease	21	6
Acute coronary occlusion	7	10
Sequelae of coronary occlusion	6	8
Cor pulmonale	2	10
Obesity	8	3
Thyrotoxicosis	2	2
Myxoedema	1	1
Uraemia	1	2
	418 pats.	679 pats.

8 patients with 43% males and 57% females, the 1964–66 group of 679 patients with 50% males and 50% females. The average age in the two groups was 68.4 years and 70.9 years.

Table II gives a comparison of the diagnoses in the two groups. It will be seen that in the 1964–66 group there is a small, but not significant, difference in the number of patients with decompensation and coronary occlusion and a considerable preponderance of patients with cor pulmonale, while there is a far smaller number of cases with atrial fibrillation and hypertension, and somewhat fewer with myocardial degeneration, mitral disease and obesity.

The incidence of digitalis intoxication during 1950–52 was 36 of 418 patients, and during 1964–66 120 of 679 patients, which gives a significant increase from 8.6% to 17.7% ($p < 0.001$). These values include only patients who have exhibited symptoms and signs of intoxication during the course of digitalis treatment in hospital, not patients admitted with symptoms and signs of intoxication due to digitalis therapy at home. The criterion of diagnosing digitalis intoxication was either 1) the symptoms and signs had to arise in distinct relation to digitalization or to an increase of a previously tolerated maintenance dose or 2) if the symptoms and signs had arisen on a previously tolerated maintenance dose, they had to cease when digitalis was withheld or the dose reduced.

There was seen no difference in the frequency of digitalis intoxication during the two periods as far as the digitalization is concerned (11.5% in 1950–52, 9.9% in 1964–66), the whole difference occurring during the maintenance therapy (4.2% in 1950–52, 14.1% in 1964–66).

Table III gives the relative occurrence of the recorded symptoms and signs of intoxication. In 1964–66 the classical symptoms (dyspepsia and visual disturbances) were fewer but electrocardiographic changes more common (89% as compared with 44%) especially in the form of atrioventricular block of all degrees and tachycardias. The number of fatal cases, i.e. cases in which digitalis was the direct or contributory cause of death, is practically the same in both groups, viz. approx. 8%.

Table III. *Percentual distribution of the signs and symptoms in the intoxicated patients*

	1950–52 (%)	1964–66 (%)
Nausea	61.7	46.7
Vomiting	44.4	30.8
Diarrhoea	27.8	24.2
Ocular signs	22.2	13.3
Cerebral symptoms	2.8	7.5
Deaths	8.3	7.5
Gynaecomastia	2.8	2.5
Urticaria	2.8	0.0
Multifocal extrasystoles	8.4	15.8
Bigeminal pulse	33.3	33.3
Trigeminal pulse	5.6	4.2
First degree heart block	5.6	25.0
Second degree heart block	2.8	12.5
Third degree heart block	0.0	5.8
Atrial fibrillation	0.0	0.8
Atrial flutter	0.0	0.8
Nodal rhythm	2.8	5.8
Paroxysmal tachycardia	0.0	4.2
Ventricular tachycardia	0.0	2.5
Brachycardia	27.8	25.0
ECG changes in all	44.4	89.2
ECG changes only	19.5	43.3
	36 pats.	120 pats.

Of course it is of decisive importance to ascertain whether the principles of digitalis therapy were uniform during the two periods. We tried to assess this by stating whether the dose for digitalization or for the maintenance therapy in each individual case was equal to, above or below a defined normal dose. A normal dose we took to be the dose most commonly used in this country with a certain margin on both sides, i.e. a dose for digitalization of 1000–1500 mg digitalis leaf and a maintenance dose of 400–600 mg weekly. Figs. 1 and 2 show the distribution of the patients according to the selected criteria. In general it may be said that in 1964–66 the dosage was more moderate, in particular for digitalization.

In Table IV the patients are divided into groups: under and over 70 years of age, compensated and decompensated. The incidence of intoxication is the same in both age groups, while it is considerably higher in decompensated than in compensated patients. Further investigation revealed that the average dose of digitalis to these four groups was the same.

The diuretic therapy is analysed in Table V. While previously mercurials were in most common use, these agents have now been almost completely replaced, primarily by chlorothiazides and chlorthalidone (Hygroton®), and to a lesser extent by frusemide (Lasix®) and ethacrynic acid (Edecrin®). During 1964–66 diuretic therapy was on the whole more common than in 1950–52. During both periods diuretics were used more for intoxicated patients. In 1964–66 moreover the intensity of the diuretic therapy was greater among the intoxicated patients, 31% of whom had received more than one diuretic as compared with only 10% in the non-intoxicated group.

Determinations of the serum potassium level had been done on so few patients in 1950–52 that these determinations were not included in the analysis. In 1964–66, on the other hand, 647 out of 679 patients had had determinations of serum potassium, the majority repeatedly. Among these patients 531 patients were not intoxicated and had an average serum potassium of 4.06 ± 0.02 and 116 patients were intoxicated and had an average serum potassium of 3.61 ± 0.05 ($p < 0.001$). The percentual distribution of patients at various serum potassium levels is plotted on Fig. 3. These calculations are based on one

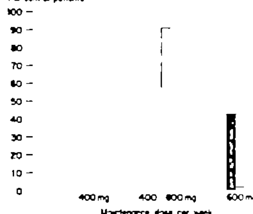


Fig. 2. Maintenance dose for 361 patients in 1950– ▨ and 665 patients in 1964–66 □.

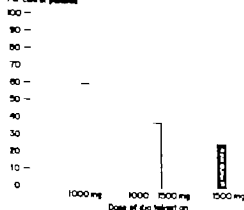


Fig. 1. Dose of digitalization for 183 patients in 1950–52 ▨, and 262 patients in 1964–66 □.

Table IV. *Patients grouped by age over and under 70 years and by decompensation and compensation*

	1950–52			1964–66		
	Total no. of pats.	Intox.	%	Total no. of pats.	Intox.	%
< 70 y	218	20	9.2	297	49	16.5
> 70 y	200	16	8.0	382	71	18.6
Decompensated	177	27	15.3	356	102	28.7
Compensated	241	9	3.7	323	18	5.6

Table V *Diuretic treatment of non-intoxicated (– Intox) and intoxicated (+ Intox) patients*

	– Intox. (%)	+ Intox. (%)	Total (%)	– Intox. (%)	+ Intox. (%)	Total (%)
Mercurial diuretics	38.5	58.3	40.2	1.8	4.2	2.2
Chlorothiazides/chlorthalidone				46.3	76.7	51.7
Frusemide				11.3	28.3	14.3
Ethacrynic acid				2.1	10.0	3.5
Combination of several of the above agents				9.8	30.8	13.5
Diuretic treatment in all	38.5	58.3	40.2	50.0	80.8	55.5
Total no. of pats.	382	36	418	559	120	679

serum potassium value from each patient, the lowest one found during the treatment period in the non-intoxicated patients and the one closest to the time of intoxication in the intoxicated patients. The measurements were done by flame photometry.

DISCUSSION

The present study thus confirmed that the number of digitalis intoxications has been increasing during the past few decades. We found the incidence of digitalis intoxication among all digitalis-treated patients in a department of general medicine to have increased from 8.6% in the early 50's to 17.7% in the middle of the 60's. This increase relates exclusively to the maintenance therapy. In respect to the symptoms and signs of intoxication during the two periods, it may be emphasized that dyspeptic symptoms have receded somewhat into the background, while cardiac arrhythmias of various kinds have become far more common. The mortality is the same, about 8%. This is, with one exception (1), at the lower end of that found by others (2, 3, 11, 14).

A more advanced age among digitalis-treated patients during the latter period is not a factor in the present material, as the incidence of intoxication was the same for patients under and over 70. Decompensation, on the other hand, greatly increases the sensitivity to digitalis. However, this does not provide the sole explanation of the difference in the number of intoxications during the two periods, in which the percentual distribution of patients with decompensation was almost identical.

Several authors have claimed that today there is a tendency to administer a more intensive and quicker digitalization than previously and that pure glycosides are being used more, rather than leaf. Neither factor has played any role in the present material for the following reasons: 1) the increase in the incidence of intoxication occurred within maintenance therapy; 2) for digitalization as well as for maintenance the doses were lower in 1964–66 than in 1950–52; 3) during both periods practically all the patients received digitalis leaf.

The only factor which appears to have altered to the same extent as the number of intoxications is the diuretic therapy. In 1950–52 mercurial diuretics were used almost exclusively and administered to 40% of the digitalis-treated patients, in the intoxicated group however to 58%. During the period 1964–66 a number of different diuretics were administered to 56% of

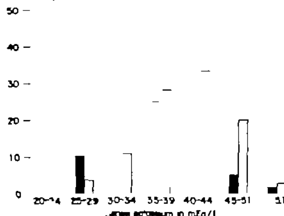


Fig. 3. The percentual distribution of serum potassium concentrations in 116 intoxicated patients ■, and 531 non-intoxicated patients □ in 1964–66.

the digitalis-treated patients (14% received more than one diuretic). Among the intoxicated patients a total of 81% had received diuretic therapy (31% more than one diuretic). This intensified diuretic therapy in the intoxicated patients must be the explanation of the difference in serum potassium concentration, viz. 4.06 mEq/l in non-intoxicated and 3.61 mEq/l in intoxicated patients in 1964–66.

It has been repeatedly demonstrated, in animal experiments as well as clinically in man that potassium depletion increases the sensitivity to digitalis. It is obvious, therefore, that any condition or any concurrent treatment tending to reduce the potassium content of the body will increase the risk of digitalis intoxication, and possibly render a previously tolerated dose of digitalis toxic. This applies to vomiting, diarrhoea cardiac decompensation, coronary occlusion treatment with corticoids, strong laxatives, and primarily to the modern diuretics (8 12). As far as the chlorothiazides are concerned, it has been demonstrated that 40% of patients treated for more than one week with chlorothiazides without receiving a supplement of potassium will develop kaliopenia (18). In this connection, the serum potassium is of value if it is reduced. The decisive factor is the intracellular potassium level, which may be reduced in spite of a normal or even elevated serum potassium (5).

These findings, combined with the change in diuretic therapy within the past 15 years entitle us, we feel, to the following assumption. By virtue of its kaliopenic affect, treatment with the modern diuretics is the main cause of the marked increase in the number of digitalis intoxications and presumably responsible for an alteration of the manifestations of intoxication i.e. tending to a lower percentage of dyspeptic symptoms and visual disturbances and to a larger number of cardiac arrhythmias.

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BLOOD FLOW IN VASCULAR DISORDERS

A Plethysmographic Study

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Abstract. The peripheral cutaneous vessels have been studied at various ambient temperatures and at local temperatures ranging from 40 to 6°C. At the higher local temperatures sympathetic stimuli exerted strong effect upon the blood flow. By applying local cooling of increasing strength the influence of sympathetic stimuli upon the vascular tone was successively abolished. From around 15°C of local temperature and down to zero vessels were unresponsive to sympathetic nerve influence. It appears that the local cold stimulus is the major determinant of the vascular tone of the skin when man is exposed to cold. Studies of the cutaneous circulation under the same conditions are carried out in patients suffering from various peripheral vascular disorders. Local cold as again found to be the determinant factor for producing the abnormal vasoconstriction. Abolition of sympathetic tone did not prevent the occurrence of spasmodic attacks. The hemodynamic difference between cold-sensitivity in vasospastic and in obstructive arterial disease of the hands is demonstrated. The findings are discussed in the light of presently applied criteria for treatment of cold-sensitive peripheral vascular disorders.

Cold-sensitive vascular disorders most often represent local manifestations of generalized disease (17), and therapy must accordingly be directed towards the latter

Sometimes, however palliative treatment becomes urgent for amelioration of painful vasospasms or prevention of tissue loss. In such cases basic knowledge of the effects of cold upon the vessels is required in orde to initiate the correct treatment.

At this point current clinical criteria are un satisfactory Sympathectomy is often recommended, although little is known of the extent to which sympathetic nerve stimuli act upon vessels exposed to cold. Similarly no clear concept exists concerning the effectiveness of general contra local protection against cold in patie t with cold-sensitive vascular disorders.

This report is a study of normal hand vessel during exposure to cold and of the individua role played by sympathetic nerve stimuli and local cold stimuli. A similar study is being carried out on hands suffering from some types of cold-sensitive vascular disorders.

MATERIAL AND METHODS

Three healthy volunteers, aged from 21 to 45 years, underwent the experiments and provided the control values.

T females, aged 4 and 36 years, are included in the study as representatives of pure vasospastic disease. They had both been suffering from severe vasospastic attacks on cold exposure for more than ten years. No underlying disease could be demonstrated, and their hand vessels proved to be patent by arteriography

Two men, aged 47 and 54 years, were studied as representatives of obstructive vascular disease. One was physician working in northern district of Norway For some months he had been seriously troubled by severe vasospastic attacks of the right hand.

When visiting patients during the winter he had to take with him bottles of hot water for soaking his hand and thus get release from the vasospasms. Angiography revealed obstruction of the main hand vessels and thrombotic occlusion of the superficial palmar arch. Some months after undergoing the present examination he became the victim of acute, bilateral thrombosis of the femoral arteries The basic metabolic abnormality was hypercholesterolemia. The other man as admitted with impending gangrene of the left hand. After some weeks of anticoagulant therapy and abstinence from tobacco improvement took place. Angiography revealed extensive obstructive arterial disease of the left hand. Hypercholesterolemia was also present in this case. Later on he suffered from coldness of the left hand and of severe vasospastic paroxysms on cold exposure.

The last patient to be included was male, 58 years

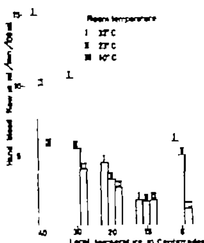


Fig. 1. Hand blood flow in normal subjects at various ambient and local temperatures.

old, suffering from high-titre cold hemaglutination (8, 14). The underlying disorder was non-syphilitic type f paroxysmal cold hemoglobinuria. He was included in the series as representative of a cold-sensitive vascular disorder due to intravascula stasis.

The normal subjects as well as the patients were examined in the supine position temperature-controlled room (9). They were naked, except for small anket over the genital area.

Hand blood flow as determined by the venous oc clusion technique in w ter filled plethysmograph at local temperatures of 40, 30, 20, 15 and 6°C

The controls were examined at room temperatures of 32, 23 and 10°C. For the patients only the highest room temperature was employed.

The examinations are unpleasant for the normal subjects and much more so for the patients, some of whom refused to complete the studies. Only those who ful filled the program ar included in this report. Several participants complained f numbness and coldness of the hands after the cold exposure. Usually the discomfort was transient. One woman, however suffered from sensory loss of the skin at the fifth finger and lateral aspect of the hand for several weeks. Such events have also been noted by other authors (4).

In addition the experiments were time-consuming and difficult to conduct. Similar experiments are therefore unsuitable for routine clinical work.

RESULTS

As expected the normal hand blood flow decreases successively on lowering of the local tem perature from 40 to 15 C (Fig. 1).

At local temperatures of 40 and 30 C the effects of various magnitudes of sympathetic nervous discharge upon the blood flow are observed to be great. At an ambient temperature of 32 C the subjects are uncomfortably warm and dissipate heat by means of cutaneous vasodilation. In contrast, at the ambient temperature of 10 C the subjects are shivering from cold and attempt to avoid any loss of heat by means of severe cutaneous vasoconstriction. At the ambient temperature of 23 C the subjects are in a state of thermal equilibrium with no need to dissipate or conserve heat. The effects upon the peripheral vessels of various ambient temperatures are thus mediated via the vasomotor centre, which regulates the vascular tone by varying the impulse rate of the sympathetic nerves.

It can now be observed that this centrally arising sympathetic nerve influence markedly declines with decreasing local temperatures and becomes lost at 15 C local temperature. Some important conclusions can now be made. First ly the local cold stimulus is the major determinant of the tone of the cutaneous vessels during cold exposure. Cold-induced peripheral vasoconstriction can consequently be reversed only by local heating. Secondly abolition of sympathetic nerve activity is unlikely to prevent pe-

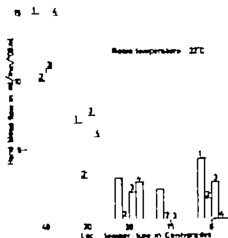


Fig. 2. Hand blood flow in various types of peripheral vascular disease t various local temperatures. 1 normal circulation. 2, vasospastic disease of the hand. 3, obliterative arterial disease of the hand. 4 hand from patient suffering from high-titre cold hemagglutination.

ripheral vasoconstriction precipitated by cold exposure

At the local temperature of 15 C the hand blood flow is at its minimum. At lower local temperatures the blood flow again shows a rise, which represents the well-known cold vasodilation. This is distinctly influenced by the ambient temperature. Apparently the sympathetic nerves have resumed their function, an observation in contrast to the above findings. However the accordance of the events is complete throughout the whole experiment. A series of brilliant studies of the cold vasodilation have proved that the vessels at this low local temperature are quite unresponsive to sympathetic stimuli as well as to the sympathetic neurohormones (4 6, 10 11).

Thus the results of the present report lead to a new concept. This concept is that cutaneous vessels during cold exposure successively become unresponsive to sympathetic stimulation, which below 15 C local temperature does not at all affect the tone of the peripheral skin vessels. Such a concept obviously has important bearings upon the present criteria for control and treatment of cold-sensitive vascular disease.

Important observations also appeared from exposing three categories of vascular disorders to the experimental procedure (Fig. 2). Unfortunately this procedure had to be restricted to only the highest ambient temperature, because the immersion of the diseased hands in the cold water caused severe discomfort and also complications. This meant that the hands during this experiment could be regarded as being free from any sympathetic vasoconstrictor influence.

The blood flow in hands with vasospastic disease is clearly subnormal at the higher local temperatures despite the lack of sympathetic vasoconstrictor stimuli. This clearly points to the presence of a local fault in the vessels. The sensitivity to local cold is also apparent in these hands by the rapid decline of blood flow on reducing the local temperature from 40 to 30 C. At 20 C the blood flow is zero so that the critical temperature for occurrence of complete vasoconstriction must be situated somewhere between 30 and 20 C. Similarly blood flow is unmeasurably low at 15 C, while these hands with vasospastic disease show remarkable cold vasodilation. As seen, abolition of sympathetic nerve stimuli could not prevent the hands with this disorder from undergoing the abnormal constriction on cold exposure Here again local cold stimulus is the determinant of vasc tone.

The hands suffering from obstructive a r disease behaved differently A subnormal b flow is present at the highest local tempe reflecting the obstructive vascular dise e contrast, the blood flow t 30 C is m above normal, as can be seen when rte struction is extensive (9). No cold-ser i observed in this temperature range d distinctly seen in the hands with sosp s ease However when lowering the loc d perature to 20 and further to 15 C uch sensitivity becomes apparent. At the lat perature blood flow is zero also in the hand fering from obliterative arterial disease mechanism behind this event must b dif from that in vasospastic disease Ag n th of sympathetic constrictor influence du n prevent the abnormal vasocon triction At the very low local temperature just bove zero distinct cold vasodilation occurred

In the hand of the patient with cold hem g glutination the vessels react quite normally t cold exposure until the critical temperature reached somewhere between 20 and 15 C l local temperature. Then intravascular clumping of red cells produces a stasis, which compl tely stops the blood flow In this case no asodila tion at 6 C local temperature ensues. As expected, abolition of sympathetic vasoconstrictor tone is useless in preventing the arrest of blood flow

COMMENTS

This study provides some essential information regarding the regulation of cutaneous vascular tone during cold exposure. Evidence is presented that the locally acting cold stimulus is the major determinant of the tone and that local cooling successively and rapidly makes the vessels unresponsive to sympathetic nerve stimuli. From local temperatures of about 15 C and down to zero the lack of response seems to be complete. These observations thus fit in with those made on cold vasodilation (4 6 10 11 13).

The reason why the local cold stimulus possesses such a dominant action upon the tone

of the cutaneous vessels is not clear. Probably a kind of counter-current principle is operating, in that cooled venous blood in returning from the hand produces a pre-cooling of the arteries (2). A vicious circle is thereby created, leaving the hand circulation beyond central sympathetic control. Augmented viscosity of the blood would work in the same direction (3, 15).

Thus it is obvious that abolition of sympathetic tone does not prevent the cutaneous vasoconstriction on cold exposure.

As also demonstrated in this report cutaneous blood flow is under powerful regulation by central sympathetic stimuli when local cooling is absent.

These facts are of essential clinical interest. In evaluating the effect of sympathectomy upon amelioration of cold-induced vasospasms it is necessary that the examinations are done at the relevant temperature of local cold exposure. Increased blood flow recorded at high local temperatures can lead to erroneous conclusions, because the local cold stimulus is the determinant of blood flow during such cold-precipitable vasoconstriction.

Similarly local cold protection remains important, except for the extreme local cooling as followed by cold vasodilation. Then the general thermal state is important (16) although not via the sympathetic nerves (10, 11).

On the other hand abolition of the sympathetic vasoconstrictor tone leads to a marked increase of skin blood flow when the vessels are relaxed or free from appreciable local cold stimuli. This is important to recognize when sympathectomy is carried out in order to heal ulcers or prevent gangrene.

In recommending sympathectomy the events reported above remain essential. The aim of the operation must be clear and the care after surgery must follow distinct rules.

Examinations of the cutaneous blood flow in patients with pure vasospastic disease proved that a local fault of the arteries was responsible for their vasospastic circulation, a finding in accordance with Lewis (1). The cold-sensitivity could be demonstrated also at higher local temperatures in contradistinction to the case in obliterative arterial disease. A distinct cold-sensitivity of the [illegible] apparent only on ap-[illegible]ion of a [illegible] cold stimulus. The cold-sensitivity and spasmodic attacks in obliterative arterial disease probably depend upon the occurrence of critical closure (1). Distal to arterial obstructions the intravascular pressure is regularly reduced. Local cooling augments the tone of the post-stenotic vessels. When this tone supercedes the intraluminal pressure, critical closure and thereby circulatory arrest is established.

Abolition of sympathetic tone did not prevent the occurrence of abnormal vasoconstriction in either type of peripheral vascular disorder a finding corroborated by clinical experience (5, 7) and also consistent with findings in this report of normal vascular tone during cold exposure.

The present study provides information of probable significance for clinical understanding of the response to cold of cutaneous vessels in man. Thus it will be helpful in arriving at the correct type of treatment.

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RESEARCH ON PERIPHERAL HEMODYNAMICS IN VARIOUS DISEASE ST[illegible]

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Abstract. By means of plethysmography at the local temperatures of 32 and 40°C [illegible] basis has been provided in terms of blood flow for distinction between vascular disorders of different etiology. This procedure of local heating produced [illegible] conspicuous fall of the blood flow in arteritis and in structural arterial disease on the border of gangrene. The significance of this observation is briefly discussed. A considerable vascular reserve capacity was demonstrated in threatening gangrene as [illegible] ell as in complete vasoconstriction. The obvious inability to put this reserve to use even under such extreme needs is commented. Examples are given of the benefits derived from a correct clinical assessment and use of hemodynamic measurements.

Disturbances of the peripheral hemodynamics most often represent local manifestations of generalized disorders (1 9 11 1[illegible], 13). It is incompletely known to what extent a distinction between these disorders is possible on the basis of hemodynamic patterns.

This report is an attempt to provide a rationale for such a distinction by a simple determination of blood flow in response to local heating. Observations are also presented on the vascular reserve in various circulatory disorders. Finally the diagnostic aid obtained from estimations of the peripheral blood flow is illustrated.

MATERIAL AND METHODS

The patients were all adult subjects referred to the Vascular Laboratory for various peripheral vascular complaints. Additional information of the patient series is given in the text.

Peripheral hemodynamics was studied in [illegible] temperature-controlled room by means of conventional plethysmography (3 6). Hand blood flow was assessed at local temperatures of 32 and 40°C, and in some experiments also at lower temperatures. Calf and foot blood flow [illegible] determined only at the local temperature of 32°C. The parameters recorded were the blood flow at rest (RBF) and the peak flow (PF) [illegible]lowing timed arrest of the circula[illegible] arrest lasted 3 min, for the ca[illegible] peak flow is also referred to as [illegible] or the [illegible]ascular capacity

RESULT

A series of essential inform[illegible] of the circulatory disturb[illegible] local heating (Table I). [illegible] should to some extent be d[illegible] ripheral blood flow is norma[illegible] magnitude in men and women (5[illegible] given are from both sexes. Attentio[illegible] centered upon the changes which th[illegible] cir[illegible] undergoes by application of local heat.

It is then observed that such a stimulus p[illegible] duces a complete normalization of the blood flo[illegible] in vasospastic states. In contrast th[illegible] augmentation of blood flow in obstructive arteria[illegible] disorders is subnormal under the same circumstances. When read from right to left, the figures indicate that cold-sensitivity is present in vasospastic states also at these high local temperatures, whereas it is absent in obstructive disease. Cold-sensitivity of the latter condition becomes apparent, however provided that actual cold stimuli are applied (4).

The hemodynamics in obstructive disease with impending gangrene undergoes a dramatic alteration during local heating. A marked reduction of the blood flow occurs. Simultaneously great pain is precipitated.

Identical events take place in the presence of arteritis with local manifestations of inflammation, phlebitis or ulceration. In widespread a[illegible]teritis without such local signs local heating most often produces a subnormal rise of the blood

Table IX. *The arterio-venous "steal effect" Foot blood flow in a woman with arterio-venous fistula of the right thigh and peripheral ischemia*

Room temperature 23°C. Local temperature 32°C

	Foot blood flow (ml, min/100 ml)	
	RBF	PF
First examination	[illegible]	4.2
Four mo. later	1.2	1.5
One mo. after operation	4.5	18.4

that blood flow could not be calculated, and simultaneously edema of the hand was noted. On the suspicion of the presence of cold urticaria the hand was immersed for 6 min in cold water (Table VI) and the diagnosis was confirmed. A slight blood pressure fall and a considerable tachycardia followed the local cold exposure. The cold allergia could be easily demonstrated by applying tubes containing cold water all over the skin and after a couple of minutes observing the typical wheals. Passive transfer was tried without success, although this usually can be produced (7). Peroral and parenteral therapy with antihistaminics and steroids was ineffective. the following months the patient became in that cold exposure brought on severe of the exposed parts such as face and hands and a cold shower usually produced numerous wheals and alarming general reactions. In the course of three years his complaints successively diminished and ultimately disappeared completely

The diagnosis of the subclavian steal syndrome" can easily be overlooked due to frank arterial pulsations and inconspicuous blood pressure reduction in the affected arm. A 57 year old carpenter complained of dizziness and cramp-like pain of the left fist during work. The blood pressure in the right and left arm was 160/95 and 150/100 respectively and arterial pulses were felt at normal strength in the left upper extremity. Plethysmography of both hands showed an insignificant difference of the resting blood flow but a distinctly reduced vascular capacity of the left hand (Table VII). Subsequent angiography disclosed an occlusion of the left subclavian artery proximal to the origin of the vertebral artery. This case demonstrates that collateral flow can be so powerful that a major artery stenosis may be completely obscured except for the presence of a reduced capacity of the vascular bed in question.

The seat of an arterio-venous fistula is usually easy to determine by means of angiography but this is not always the case. A 52-year-old man presented with obvious symptoms of an arterio-venous fistula of the left leg. Angiography did not produce convincing evidence of such a lesion nor of its possible position. A continuous murmur was heard over the whole popliteal space. Plethysmography of the calf tissue revealed a huge flow compatible with the diagnosis (Table VIII). The amount of calf tissue enclosed in the plethysmograph was 1390 ml. The flow through this part of the leg was thus 2.5 l/min against the normal value of 49 ml/min. Successful reconstructive surgery was later carried out by removal of a fistula at the left peroneal artery. For the surgeon the preoperative blood flow studies provide the essential information on the size of the fistula and the necessity of operation.

Sometimes a related problem arises when a proximally situated fistula possesses a steal effect so that a dangerous peripheral ischemia occurs. A 32-year-old woman presented with an arteriovenous fistula at the thigh following a stab wound twenty years earlier. Angiography and other examinations proved the fistula to be small, and immediate surgery was therefore considered unnecessary. Her real complaints, however consisted in intermittent claudication of the foot and calf coldness and ultimately rest pain. The blood flow studies (Table IX) disclosed a reduced resting flow and a largely reduced capacity of the circulation through the left foot. This insufficiency was found to be progressive and corrective surgery was consequently carried out with success.

COMMENTS

Measurements of peripheral circulation provide two flow variables. One is the resting blood flow which serves to cover the immediate need for tissue oxygenation. The other is the hyperemic flow provoked by a standard stimulus and which reveals the patency of the vascular bed and its

capacity to increase its blood flow The present report shows how a systematic use of these variables can give essential information upon problems in circulatory pathophysiology

By estimating the resting blood flow in response to a simple procedure of local heating, an etiological distinction between various kinds of circulatory disorders can be made.

The cold-sensitivity of vasospastic disease demonstrated at lower local temperatures (4 10) is *in fact demonstrable over a great spectrum from* 15 to 40 C.

In contrast the cold-sensitivity in structural arterial disease is only demonstrable in the lower range of that interval of temperatures (4). Exact knowledge on this point is essential for detecting atherosclerotic disease at an early stage or as a concomitant disorder in, for instance, hypertension (13).

The adverse effect of local heat upon blood flow in arteritis and in impending gangrene, as demonstrated in this report, is of essential interest. The pain released by local heating has formerly been ascribed to the occurrence of increased tissue metabolism in the presence of a restricted but constant blood flow The observations presented indicate that pain results from a simultaneous increase of tissue metabolism and decrease of blood flow It is unlikely that pain is caused only by the latter because treatment with phenoxybenzamine under similar circumstances (6) precipitated blood flow reduction in the absence of pain.

The exact cause of such an adverse effect of heat cannot be given. It is, however probable that nervous pain reflexes play significant role. Circulation in impending gangrene is maintained mostly by collaterals and the perfusion pressure is low Even a small increment of nervous tone may suffice to precipitate critical closure and a sort of vascular collapse.

As regards the other flow variable, the capacity flow or the flow reserve, new information is also presented. The concept of maximal vasodilation in impending gangrene must be revised, as a considerable vascular reserve has been shown to exist in this condition. Likewise a reserve capacity has been demonstrated in vascular beds under complete vasoconstriction. Obviously the vascular capacity as estimated by the hyperemic response is flow variable, which the circulation is unable to draw upon even under critical circumstances.

These observations have to be considered also for other vascular areas of the body It can not be excluded that fever may deteriorate myocardial flow for instance in ischemic heart disease.

Although the circulation of the human skin seems to be unable to draw advantage from its reserve capacity the latter flow variable is very useful *for diagnosis and follow-up of vascular* disorders. A few examples are presented to illustrate the benefits derived from flow studies in clinical work.

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FAILURE OF CONVERSION OF ATRIAL FIBRILLATION

Relation to Fibrillatory Wave Size

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Abstract. Up to 1969 there were in the Department of Medicine, University Hospital, Uppsala, 42 patients who failed to convert from atrial fibrillation to sinus rhythm in the countershock procedure. It has not been possible to separate this series of patients from reference material as regards the fibrillatory wave size.

All reports on direct current conversion of atrial fibrillation are characterized by an extremely high convertibility rate (3, 6, 7). The failures are mainly seen in the group of patients with a long-standing arrhythmia (3, 6). No other reliable characteristics of the failure group have been universally accepted.

A few authors have attempted to correlate the success of the conversion procedure to the size of the *f* waves (7, 8).

The purpose of this report is to study the *f* wave size within the patient group of atrial fibrillation not converting in the countershock procedure.

MATERIAL AND METHODS

Since 1963 conversion of atrial fibrillation has been performed with the direct current countershock technique at the Department of Medicine, University Hospital of Uppsala. The procedure as used by us has earlier been described (2, 3).

The present study includes all patients who have failed to have conversion of their atrial fibrillation.

Up to 1969 the total number of patients with such failures was 42. A review of the etiologic diagnoses in this group is shown in Table I. It should be stressed that, in our material of patients undergoing conversion attempts, there is an overrepresentation of atrial fibrillation due to valvular heart disease. This seems primarily due to the presence of an active thoracic surgery group within the hospital.

In the pertinent patient series, the *f* wave amplitude was determined on all ECGs available from the time prior to the conversion failure. The *f* wave size was usually measured in lead V or CR_1, where the atrial activity was most easily observed.

The grouping of the *f* waves was done in accordance with previous work in the field (1, 5, 8). Mostly the *f* waves were easily classified and in borderline cases the most common *f* wave size of the ECGs of each patient was the decisive factor for the definite grouping.

All patients were having maintenance dosage of digitalis at the time of *f* wave measurement.

The electrocardiographs used were Elema-Schönander (Elema-Schönander Ltd., Stockholm) direct-writing machines, mainly Mingograph 42 (linearity ±10 mm and flat frequency response up to 500 /sec). The vast majority of the ECGs were recorded with paper speed of 50 mm/sec.

As reference material I have used an earlier study of my own of the *f* wave size in an unselected sample of patients with atrial fibrillation (1).

RESULTS

The different f wave sizes in patients where conversion of atrial fibrillation has failed is shown in Table II. In Fig. 1 the ratio, in percentage, of the individual *f* wave group to the total material is given. The two series are almost identical as regards the aspect under study.

DISCUSSION

In 1962, Aber (4) presented a paper on the value of quinidine after mitral valvotomy. A part of his investigation dealt with different atrial patterns defined as type I–IV. The type I pattern had no atrial baseline oscillations, and type IV had very regular *f* waves and waves of large amplitude. However he declared that the patients were in broad groups and were not in-

Table I. *Etiology and age in the failure group*

Etiology	No of pats.	Mean age (y.)
Valvular heart disease	27	50.7
Non-valvular heart disease	13	61.5
Miscellaneous[a]	2	62.5
Total	42	54.6

[a] Two patients with treated hyperthyroidism.

Table II. *The distribution of patients with different f wave sizes*

Etiology / f wave size, mm	No. of pats. 0–0.5	0.5–1	1–2.5	>2.5
Valvular heart disease	2	16	8	1
Non-valvular heart disease	5	4	4	
Miscellaneous[a]	1	1		
Total	8 (19)	21 (50)	12 (29)	1 (2)

Numbers within brackets, per cent of whole material.
[a] Two patients with treated hyperthyroidism.

tended to represent ranges of precise measurements"

He found that patients with atrial patterns of type I and II had a 100% failure in reverting rhythm, whereas 75% roughly reverted type III and IV atrial patterns.

This interesting finding has later been discussed as regards convertibility rate using direct current countershock technique.

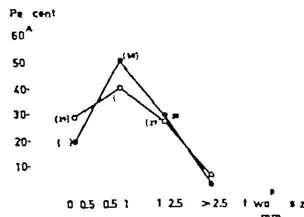


Fig. 1. Distribution of patients with different *f* wave sizes in per cent of the total patient series. Filled circles, failure group. Open circles, reference material. Numbers within brackets, per cent of total.

Thus, Oram and Davies (8) as well as Lown (7) have confirmed the same finding as Aber (4) made at the end of the quinidine era.

Oram and Davies found that sinus rhythm was more easily established as well as maintained in patients with coarse atrial fibrillation. This finding was not significant at the 5% level, however.

Lown (7) reported that patients with fine atrial fibrillation (amplitude less than 1 mm in V_1) required more energy to be converted and also had a higher per cent of failures. In this group the mean conversion energy in 50 patients was 140 Ws and there were 20% failures. In another 50 patients with large *f* waves (more than 2 mm in V_1) the conversion energy was 92 Ws and there were only 4% failures. He also found that the *f* wave size was inversely related to the duration of the atrial fibrillation, i.e. the longer the duration the smaller the *f* waves. Therefore, his suggestion was that the factor of the greatest importance, when evaluating convertibility having regard to *f* wave size, was the relationship between the *f* wave and the duration of the arrhythmia.

Comparing the series of Oram and Davies (8) with our own, there are some observations to be made. In their series the composition of different *f* waves is dissimilar to that in my series. Oram and Davies report 33% to be 1 mm or less in their studies, whereas the corresponding figures were 69% in the failure group and 61% in the reference group. A possible explanation of this might be the overrepresentation of patients with fine atrial fibrillation in the failure group. The almost identical percentages in the failure group and in the reference material are then more difficult to accept, as the reference material should be an unselected sample of patients with atrial fibrillation. On the other hand the mean age in the necropsy material is higher than in a clinical material such as that of Oram and Davies. Aravanis et al. (5) have proposed that the *f* wave amplitude decreases with increasing age of the patient. In my series, however, I have not found this trend at increasing age of the patient (1). On the contrary the *f* wave pattern seems to be comparatively stable in the individual case, for which reason I do not feel that this is the crucial explanation.

In other words, there was a failure of predict

ing the outcome of attempts at conversion from the measurement of *f* wave amplitude.

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MAINTENANCE OF SINUS RHYTHM AFTER CONVERSION OF ATRIAL FIBRILLATION

Relation to Fibrillatory Wave Size

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Abstract. In the Department of Medicine, University Hospital, Uppsala, series consisting of 35 patients maintained sinus rhythm for more than one year after DC conversion of atrial fibrillation. The actual *f* wave amplitude prior to the pertinent conversion was determined. The composition of the different *f* wave sizes in this group as the same as in an unselected sample of atrial fibrillation. The long-term prognosis after conversion of atrial fibrillation with regard to maintenance of sinus rhythm, cannot be predicted from the *f* wave amplitude in this series.

It would be very interesting and valuable if the outcome of the conversion of atrial fibrillation could be determined from the size of the fibrillatory waves (*f* waves). In the literature it has been said that the greater the *f* wave amplitude the better the immediate result at regularization of the fibrillation (9 10). To my knowledge a study of *f* wave size with regard to maintenance of sinus rhythm has not been reported previously and this was the purpose of this publication.

MATERIAL AND METHODS

Since 1963 conversion of atrial fibrillation has been performed with the direct current countershock technique at the Department of Medicine, University Hospital of Uppsala. The method has earlier been described from our Department (3, 4).

In this study 35 consecutive patients were selected who maintained sinus rhythm for more than one year after conversion. A review of the etiologic diagnoses is shown in Table I. The patient with atrial fibrillation without any well-defined etiologic diagnosis has been referred to the non-valvular group.

In the patient series the *f* wave amplitude was measured on all ECGs available from the time before conversion. The measurement was made in that lead here the atrial activity was most clearly visible. As rule this was in lead V or CR_1. The electrocardiographs were direct-writing machines from Elema-Schönander (Elema-Schönander Ltd., Stockholm) The vast major of the ECGs were recorded with Mingograph 4 line ty ±10 mm and flat frequency response up to of /sec). The paper speed used as 50 mm sec

The grouping of *f* waves as done in accordance with other work in the field (1, 6, 11 12, 13) All patients ere on maintenance digitalis therapy hen their *f* aves were measured.

As reference material I have used my own earlier study of *f* waves (1).

RESULTS

In Table II the results of the *f* wave study are given. Fig. 1 shows the results compared with those from an unselected population of atrial fibrillation (1). There is no significant difference between the two series. In other words the group selected as having a fairly good prognosis, with regard to maintenance of sinus rhythm post conversion, does not deviate from the *f* wave size in an unselected sample of patients with atrial fibrillation.

DISCUSSION

A more distinct characterization of *f* waves was first reported by Hewlett and Wilson in 1915 (8). Cookson (7) was the first to discuss the *f* wave size with reference to different etiologies of atrial fibrillation. Thurmann and Janney Jr (1) separated the *f* waves into fine and coarse. The clinical significance of these are still not known, although some authors have correlated their size to etiologic diagnosis of atrial fibril-

Table I. *Etiology and mean age of the group under study*

Etiology	No of pats.	Mean age (y)
Valvular heart disease	21	47.0
Non-valvular heart disease	10	60.8
Miscellaneous[a]	4	45.5
Total	35	50.8

[a] Three patients had myocarditis, and one was operated upon for a coronary artery fistula.

Table II. *The distribution of patients with regard to different f wave sizes*

Etiology f wave size, mm	No of pats. 0–0.5	0.5–1	1–2.5	>2.5
Valvular heart disease	4	8	7	2
Non-valvular heart disease	3	6	1	
Miscellaneous	1	1	2	
Total	8 (23)	15 (43)	10 (28)	2 (6)

Numbers within brackets are per cent of the total material.
Three patients had myocarditis, and one was operated for coronary artery fistula.

lation or to the degree of atrial enlargement (6, 7, 12).

The author has recently shown that it is probably more valuable to study *f* wave frequency instead of amplitude. Thus, it has been shown that atrial fibrillation due to non-valvular cause is more irregular and has a higher frequency compared to atrial fibrillation in patients with pure rheumatic valvular disease (2).

During the last few years the *f* wave size has sporadically been discussed in connection with the outcome of conversion of atrial fibrillation. In 1962 Aber (5) presented a paper on *f* wave size related to the success of quinidine conversion. The patients were collected from a thoracic surgery department, and the group consisted of patients with mitral stenosis. Aber reported that quinidine conversion was more easily obtained in cases of atrial fibrillation with a more prominent *f* wave amplitude. The same statement has been made when attempting to give the immediate prognosis of DC conversion of atrial fibrillation (9, 10). Thus, in 1964 Oram and Davies (10) found that patients having atrial fibrillation with coarse *f* waves were more easily converted than those with fine *f* waves. Their results, however, were not significant at the 5% confidence level.

Lown holds a similar opinion in his Thomas Lewis lecture in 1965 (9). This opinion also sounds logical because of the very favourable experience with DC conversion of atrial flutter where the atrial activity has a more prominent magnitude. Although suggestive, the difference in mechanism between atrial flutter and fibrillation probably overshadows the similarity of atrial activity amplitude on scalar ECG.

In the present study the author did not find any correlation between the maintenance of sinus rhythm after conversion of atrial fibrillation and the *f* wave amplitude. This statement is based on the comparison with a previous study of *f* wave amplitude in an unselected sample. A few remarks on such an opinion should be made. First, there is a difference in age between the clinical material in the present study and in the reference material. Some authors have noted a decrease in *f* wave size with increasing age (6). Second, in the present study the patients were all on maintenance digitalis therapy. However, in a previous study the *f* wave amplitude was only exceptionally changed by digitalization on scalar ECG (1).

In spite of these two factors of uncertain significance, when comparing the two series I feel that it is safe to conclude that the long-term prognosis after conversion of atrial fibrillation cannot be predicted from the size of the *f* waves.

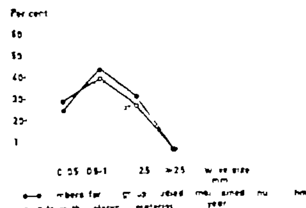


Fig. 1. The separate grouping of patients with different *f* wave sizes in per cent of total patient series.

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ISOTOPE RENOGRAPHY COMBINED WITH RECORDING OF ISOTOPE CYSTOGRAM IN PATIENTS WITH RENAL TRANSPLANTS

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Abstract. ¹³¹I-hippuran renography combined with recording of an isotope cystogram has been carried out in 23 patients who had received a renal transplant. Altogether 120 renographies were performed. The control series consisted of nine patients with stable renal function 68 to 720 days after transplantation. With acute as well as with chronic rejection an elevation in the third phase of the renogram was seen. An excretion ratio was calculated; this was significantly different from the excretion ratio found in the control series. The renogram became normal pari passu with normalization of renal function in patients with acute rejection. In three patients with obstruction of urinary outflow a reduced excretion ratio was found, accompanied by an increased transit time. The transit time in patients with acute or chronic rejection did not differ from that found in the control patients. It was thus possible in these three cases to differentiate between acute rejection and obstruction by the use of renography combined with isotope cystography.

Isotope renography (isotope nephrography) refers to a graphic registration of changes in radioactivity over the kidneys on external measurement after intravenous injection of an isotope tagged substance excreted by the kidneys. Isotope renography after administration of ¹³¹I-hippuran has been used in the study of renal function after renal transplantation in man (1, 2, 3, 6, 8, 13, 17) and in animals (4, 5, 9, 14). It has been shown in both clinical and experimental studies that threatening rejection of a transplanted kidney is accompanied by a reduction in the ability of the transplant to concentrate and excrete ¹³¹I-hippuran (1, 2, 3, 5, 6, 8, 9, 13, 14, 17).

The earliest and most constant changes have involved a reduced ability to excrete the ¹³¹I hippuran which has been absorbed resulting in an elevation of the third phase of the renogram (6, 9, 13, 17). This defective ability to excrete hippuran is commonly accompanied by delayed appearance of the third phase of the renogram (9, 13, 19).

The purpose of our studies has been to elucidate

1. what changes appear in the renogram after renal transplantation,
2. to what degree these changes can aid in the early diagnosis of rejection and
3. whether differentiation between obstruction and rejection can be made with renography

MATERIAL

Twenty-three patients who had received a kidney transplant were studied with a total of 120 renograms. These studies were done in the period November 1967 to November 1968. Eight patients had received a renal transplant prior to the period of study. The other 15 received a renal transplant during the study period. A series of renograms, consisting of 4 to 12 tracings, were carried out in 12 patients; in the other 11 patients 1–4 tracings were performed.

The patients were divided into the following groups.

Group I

Nine patients with stable renal function 68–720 days after renal transplantation (Table I).

Group II

Seven patients in whom renography was carried out during the first post-transplant week. Acute rejection was diagnosed 2 to 5 days after operation in all these patients. The donor had been a closely related family member. The ischemia period in association with transplantation varied from 31 to 72 min, and urine production was observed a few minutes after establishment of

Table I. *Kidney transplants Stable kidney function*

Pat. no.	Age	Renography days post-transplant	C_{cr} (ml/min) Average of 3 measurements	UR (2.5)	ER (15)	Urine flow during renography (ml/min)	Transit time (min)
400	27	320	90.0	1.68	1.33	7.0	2.75
403	40	720	100.0	1.76	1.71	2.3	3.10
404	27	276	80.0	1.68	1.54	1.2	3.70
411	32	383	80.0	1.64	1.41	2.6	4.00
416	26	322	70.0	1.58	1.37	4.8	2.50
428	60	220	60.0	1.55	1.27	3.4	3.70
436	31	261	50.0	1.56	1.34	0.8	5.10
443	34	242	70.0	1.66	1.47	0.8	4.30
479	16	68	90.0	1.84	1.62	3.1	3.00
Average			77	1.66	1.45	2.89	3.57
S.D				0.10	0.15		0.83

the vascular anastomoses (Table II). The possibility that ischemic damage taking place during transplantation could affect the renographic picture in these patients is discussed.

Group III

Eight patients in hom clinical and biochemical studies suggested acute rejection. With the exception of two cases this diagnosis was made more than two weeks after transplantation and renography was in all cases ed more than one week after transplantation. For this reason ischemic damage occurring in connection with the transplant operation itself could be ruled out as a factor influencing the renographic tracing. The diagnosis, acute rejection, was made on the basis of a varying number of the following signs or symptoms: fever malaise, tenderness about the graft, leucocytosis, eosinophilia, decreasing renal function ith retention of ater and sodium, hematuria, increasing proteinuria and lymphocyturia (16, 18). Renography was performed from 0 to 4 days after the first evidence of rejection had appeared (Table III).

Group IV

Five patients with chronic rejection. The diagnosis had been made on the basis of a progressive deterioration of renal function. In all cases the diagnosis of chronic allograft rejection was verified histologically on renal biopsy or at autopsy (Table IV).

Group V

This group included three patients with signs of obstruction (Table V).

Seven patients, nos. 420, 436, 443 461 479 484 and 492, appear in more than one group.

METHODS

Measuring apparatus

The measuring apparatus consisted of three scintill- tion crystals (3" 3") placed in lead collimator of cylindrical form with a lumen of 3" corresponding to 8.2 cm and with walls which were 1.5 cm in thickness. The crystal was recessed 10 cm from the aperture o

Table II. *Kidney transplants Renography in first week after transplantation*

Pat. no.	Age	Duration of ischemia (min)	Urine flow after (min)	Rejection day post transplant	Renography days post transplant	C_{cr} (ml/min)	UR (2.5)	ER (15)	Urine flow during renography (ml/min)	Transit time (min)
420	27	45	2	2	3	21.0	1.31	1.10	0.6	–7
436	31	75	3	2	3	11.5	1.55	0.91	0.7	5 0
443	34	48	5		4	48.0	1.63	0.85	0.5	—
462	4	42		2 3	2	81.0	2.04	1.05	1.5	6
479	16	31		2 5	5	67.0	1.41	0.93	1.4	2 0
461	21	72		2	7	70.0	1.28	1.40	1.5	–3
470	43	50	3	2	7	60.0	1.46	1.19	0.6	3 7
Average							1.56	1.07	0.97	–6
S.D							0.26	0.19	0.47	1 19

Table III *Kidney transplants. Acute rejection*

Pat. no.	Age	Duration of ischemia (min)	Urine flow after (min)	Rejection days post transplant	Renography days post transplant	C_{Cr} (ml/min)	UR (2.5)	ER (15)	Urine flow during renography (ml/min)	Transit time (min)
396	47	51	2	18	18	35.0	1.42	1.29	0.9	4.0
420	27	45	2	5	9	15.0	1.42	1.04	1.3	3.0
420	27	45	2	25	27	28.0	1.46	1.05	1.3	3.4
443	34	48	5	25	28	63.0	1.81	1.13	1.7	—
461	21	72	2	38	38	35.0	1.64	1.03	1.8	4.4
470	43	50	3	23	23	25.0	1.69	1.01	0.8	4.0
470	43	50	3	37–38	37	56.0	1.78	1.03	0.9	4.0
470	43	50	3	37–38	38	32.0	1.97	1.11	0.6	4.0
470	43	50	3	43	43	14.0	1.66	0.96	0.6	4.6
484	30	89	2	7	8	50.0	1.61	1.10	1.0	3.4
492	29	36	2	15	15	70.0	1.58	1.14	1.2	4
492	29	36	2	47	47	14.1	1.56	0.96	1.2	5.4
398	28	—	–	225	226	43.0	2.75	0.88	—	—
Average						37.7	1.73	1.06	1.11	87
S.D.							0.34	0.10	0.39	0.81

the collimator. A measuring unit was built up by means of [illegible]. Each reading took place with the use of punched paper tape. All information was processed in an electronic data processing unit (15).

Test substance

¹³¹I-hippuran from Amersham Radiochemical Centre, England, was stored in the dark at 4°C for a maximum of three weeks.

Procedure of study

Before performing a renogram, the sensitivity of the apparatus was studied by placing a standard containing known amount of test substance in a phantom under the three collimators. The patient was given 100 to 200 ml of water to drink a half-hour before the study in order to assure an adequate diuresis; 15 to 25 μC of the test substance was injected into a cubital vein in the course of 15 sec. During the first two minutes of the study counts were taken at two-second intervals, in the following 24 to 30 min at ten-second intervals. The urine was collected and the minute volume calculated.

The graft was located in the right or left iliac fossa in all patients and thus measurements took place with the patient lying flat in the supine position, usually on

Table IV *Kidney transplants. Chronic rejection*

Pat. no.	Age	Renography days post-transplant	C_{Cr} (ml/min)	UR (2.5)	ER (15)	Urine flow during renography (ml/min)	Transit time (min)
398	28	300	19.0	1.50	1.10	0.9	4.5
—	—	307	—	1.49	1.05	1.6	4.5
—	—	335	8.8	1.31	0.96	1.0	5.5
420	27	66	9.5	1.65	0.99	1.0	4.9
—	—	84	14.9	1.65	0.94	1.3	4.5
461	21	101	17.0	1.48	1.03	0.9	5.6
—	—	125	12.5	1.68	1.05	1.9	3.0
—	—	127	11.4	1.47	1.04	1.4	4.2
—	—	210	—	1.23	1.00	0.7	—
477	38	38	9.4	1.42	0.99	1.0	—
—	—	52	6.4	1.56	0.93	1.0	—
491	42	66	25.0	1.39	1.02	1.6	3.6
—	—	71	21.0	1.44	1.03	1.6	3.6
—	—	127	15.0	1.19	1.09	2.2	3.6
Average			14.16	1.46	1.02	1.29	4.32
S.D.				0.15	0.049	0.44	0.83

Table V *Kidney transplants with evidence of obstruction*

Pat. no.	Age	Renography days post transplant	C_{Cr} (ml/min)	UR (2.5)	ER (15)	Urine flow during renography (ml/min)	Transit time (min)	Intravenous urography
484	30	31	80	1.68	1.10	1.3	14.5	Slight dilated pelvis.
484	30	62	80	1.64	0.91	—	16.0	Contorted ureter Appearance of contrast in the bladder after about 15 min
492	29	148	20	1.38	0.95	1.5	16.0	Stenosis of the ureter and marked hydronephrosis
562	26	31	62	1.69	0.97	1.6	13.0	Stenosis of the ureter and marked hydronephrosis
Average				1.60	0.98	1.46	14.88	
S D				0.15	0.082		1.44	

an examining table, but in several cases in bed. If urography was available, the localization of the graft was outlined on the surface of the abdomen. If not, then the position of the graft was determined by palpation. A mark was made inside the outline of the graft so that the collimator could be aimed at the same spot from study to study. Collimator I was placed above the graft, pointing proximally at an angle of 10 to 15 degrees. Collimator II was placed above the symphysis pubis, pointing distally at an angle of 10 to 15 degrees. Collimator III was placed over the heart. All of the collimators were positioned 1 cm above the skin.



Fig. 1. Uptake ratio UR (2.5) in relationship to excretion ratio ER (15) in patients with varying levels of renal function. ○ patients with stable graft function; ●, patients with acute rejection; △, patients with chronic rejection; ▲ patients with obstruction.

Calculations

Tracings were made over the kidney, bladder and heart. In the present study only the tracings obtained over the kidney and the bladder were used and the following calculations made:

The renogram curve is usually described as consisting of three segments.

Phase I includes the first sharp upstroke of the curve and represents filling of the large vessels by the tracer substance, background activity and the beginning tubular absorption of the substance. Phase I continues directly into a slower rising part of the curve, *phase II* which represents continued tubular concentration of the tracer substance (10, 19). In order to obtain a mathematical expression for the rise of phase II we used the ratio UR (uptake ratio) (2.5):

$$\text{UR (2.5)} = \frac{\text{2.5 min value (\% of administered dose)}}{\text{1.0 min value (\% of administered dose)}}$$

When the curve has reached maximum height, the descending phase, *phase III*, begins (evacuation phase, excretion phase), which corresponds to movement of activity away from the measurement field of the collimator as activity passes via the ureters to the bladder. An excretion ratio, ER (15), is calculated as an expression of this decline.

$$\text{ER (15)} = \frac{\text{15 min value (\% of administered dose)}}{\text{25 min value (\% of administered dose)}}$$

The isotope cystogram, i.e. *the bladder curve*, is low at the beginning of the study corresponding to the background activity but begins to ascend as soon as the first activity reaches the bladder. The time from the injection of tracer substance, to the first appearance of activity in the bladder is called the transit time.

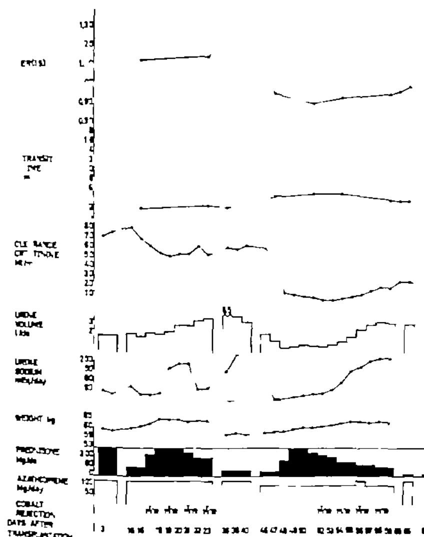


Fig. 2. Patient no. 492. The clinical course in 29-year-old man who received donor kidney from his father on May 15, 1968. During the first two post-transplant months three acute rejection episodes were seen which brought about an irreversible reduction in the function of the graft. On renography low ER (15) as found, whereas the transit time was normal. Urography 118 days post-transplant revealed ureteral stenosis and hydronephrosis. Ureteral transposition was carried out and resulted in normalization of urinary flow.

RESULTS

Group I (Table I)

In these nine patients with stable renal function 68 to 720 days after transplantation, the UR (2.5) was found to vary from 1.55 to 1.86 with a mean of 1.66. The ER (15) varied from 1.27 to 1.71 with mean of 1.45. Transit time varied from 2.5 to 5.10 min (Figs. 1, 3 and 4).

These values will henceforth be used as normal values, as we do not consider it justified to use normal values obtained from a control group of individuals with normally functioning and anatomically normally positioned kidneys. However it should be noted that the parameters given for group I lie in the same range as those obtained in individuals with normally functioning, anatomically normally placed kidneys.

Group II (Table II)

Patients in whom renograms were obtained during the first week after transplantation. In all

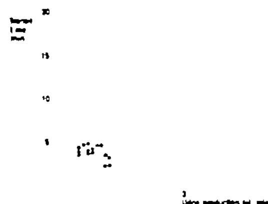


Fig 3 The relationship between the urine volume per minute and transit time in patients with renal graft. o patients with stable renal function ●, patients with evidence of acute rejection Δ, patients with evidence of chronic rejection; ▲ patients with evidence of obstruction. Transit time in patients with evidence of obstruction is obviously different from transit time in the other groups.

seven patients clinical and biochemical evidence of acute rejection appeared 2 to 5 days after transplantation. Here we found the ER (15) reduced to a mean value of 1 07 The UR (2.5) was normal with a mean of 1.56. This corresponds to the findings in patients with acute rejection (group III) Because of the short interval of time between transplantation and renography in these patients, 2 to 7 days, it cannot be ruled out that the changes demonstrated were due to ischemia.

Group III (Table III Figs. 1 2 3 and 4)

Eight patients with clinical and biochemical evidence of acute rejection. Renography was performed from 0 to 4 days after the first evidence of acute rejection had appeared.

This group was characterized by a low ER (15), mean value 1 06– This value is significantly different from the ER (15) of the control group $0.01 > p > 0.001$ (Wilcoxon test). The UR (2.5) did not differ significantly from the UR (2.5) of the control group Neither was the transit time in group III different from that in the control group (Fig. 3). As shown in Tables I and III the ER (15) was the only parameter in renograms obtained during episodes of acute rejection that significantly differed from the parameters found in the control patients. As the rejection episode was brought under control the ER (15) became normal synchronously with the other parameters used in evaluation of the effect of therapy (Fig. 2)

Group IV (Table IV Fig 1)

Includes five patients with chronic rejection. Table IV shows the primary as well as the systemic studies. A low ER (15) was characteristic of this group which had a mean ER (15) of 1.02. This value differs significantly from the ER (15) found in the control group $0.01 > p > 0.001$ (Wilcoxon test). The UR (2.5) did not differ significantly from that of the control group. The transit time was slightly longer than in the control group mean 4.32 min, but the urine volume per minute was also lower than in the control group mean 1.29 ml as against _89 ml.

Group V (Table V Figs 2 3 and 4)

Includes three patients with urinary tract obstruction The renographic characteristics were low ER (15) and markedly prolonged transit time. The ER (15) averaged 0.98 transit time 14 89 min. The UR (2.5) was normal.

Patient no 484 is a 29 year-old woman who received a donor kidney from her mother on May 8, 1968 Acute rejection episodes were



Fig 4 Transit time in relationship to excretion rate ER (15) in patients with varying level of renal function. o, patients with stable renal function, ● patients with evidence of acute rejection, Δ, patients with evidence of chronic rejection; ▲ patients with evidence of obstruction.

diagnosed on the second and eighth post-opera tive day. With an increase in immunosuppressive therapy and cobalt irradiation, evidence of re jection disappeared and the renal function became stable. Thirty days after transplantation infusion urography showed a slightly dilated renal pelvis and delayed contrast excretion in the bladder. On renography a markedly prolonged transit time and a low ER (15) were found. The slight hydronephrosis persisted for 30 days, whereafter the renographic as well as the roentgenographic signs of obstruction disappeared. The renal function was not affected.

Patient no. 492 is a 29-year-old man who re ceived a donor kidney from his father on May 15 1968. During the first two months after transplantation, three acute rejection episodes were diagnosed and an irreversible reduction in the function of the graft took place. The renographic picture was marked by a low ER (15), whereas the transit time was normal. Fifty-eight days after transplantation intravenous urography showed no evidence of obstruction. On intravenous urography 118 days after transplantation, stenosis of the ureter and marked hydronephrosis were found. Renography revealed a low ER (15) and an increased transit time (Fig. 2).

Patient no. 493 is a 26-year-old woman who received a donor kidney from her sister on October 16 1968. Postoperatively there was a good renal function and no evidence of rejection. Eighteen days after transplantation infusion urography showed ureteral stenosis and marked hydronephrosis. A renogram revealed a low ER (15) and an increased transit time.

In the latter two patients evidence of obstruction was absent after ureteral transposition and resection of the stenosis, respectively.

The characteristic findings in these three patients with evidence of obstruction were: reduced ER (15), markedly prolonged transit time and normal UR (2.5). The only feature which renographically distinguished these patients from the group with acute rejection was the prolonged transit time (Tables III and V and Figs. 2, 3 and 4).

DISCUSSION

The most important complications threatening the graft after renal transplantation are acute rejection which may lead to destruction of the graft, and acute obstruction which carries the risk of graft rupture or necrosis of the anastomosis between the graft ureter and the recipient's bladder or ureter.

The potential therapeutic possibilities for stopping and reversing an acute rejection episode are very good, and thus it is desirable to recognize this condition as early as possible. Vigorous clinical observation of the patient together with daily laboratory analyses are still the most important prerequisites for making the diagnosis of acute rejection. We feel, however that renography can be a useful adjunct to the other studies in diagnosing acute rejection. Alterations in the renogram characterized by a reduced ability to eliminate ^{131}I-hippuran appear early in the course of rejection. These alterations gradually remit as rejection is brought under control. Normalization of the renogram may thus be an expression of the effect of therapy. This defective ability to eliminate ^{131}I-hippuran, in the present work expressed by a reduced ER (15), which is seen with rejection, is not pathognomonic for that condition, as it may also be seen with ischemic renal damage and with obstruction. This change in the ability to eliminate the tracer substance seen with rejection has also been explained on the basis of partial obstruction of the ureter caused by ureteral edema as a result of rejection (5). Autoradiographic studies in rabbits after the administration of ^{131}I-hippuran during rejection of a renal transplant make this theory unlikely (9). The autoradiographic and renographic changes seen during rejection and those produced by ischemic damage are quite similar (9). The tubular cells continue to take up ^{131}I-hippuran, whereas transport to the tubular lumen is slowed because of a reduced tubular urine flow (7, 9, 13). In the seven patients in group II in whom renography was performed 2 to 7 days after transplantation, there was clinical and biochemical evidence of acute rejection 2 to 5 days post-transplant. Renographically this group could not be differentiated from group III. It is possible, however, that these renographic changes either completely or partly could have been the result of ischemic damage to the kidney as a consequence of the transplantation operation itself. Others feel that, with frequent renograms during the first post-operative days, it is

possible to differentiate the changes due to rejection from those caused by ischemia in connection with transplantation (13).

As is evident from the present study it is possible to differentiate rejection from obstruction with the aid of renography combined with registration of the isotope cystogram. Transit time in all patients with acute and chronic rejection was not essentially different from transit time in patients with stable graft function. The transit time in patients with obstruction was, however markedly prolonged. But it should be emphazized that, in patients with anuria or sharply reduced urine flow (less than 0.2 ml/min) caused by rejection or nearly complete obstruction, isotope renography is of no value in defferentiating between obstruction and acute rejection.

Renography combined with registration of the isotope cystogram is a rapid and simple procedure which demands no preparation of the patient. It gives valid information as to graft function and to the possible presence of obstruction, and thus this procedure is a useful supplement in evaluation of graft function in both hospitalized and non-hospitalized renal allograft recipients.

ACKNOWLEDGEMENT

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PRIMARY LYMPHOEDEMA COMBINED WITH HEREDITARY RECURRENT INTRAHEPATIC CHOLESTASIS

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Abstract. Five related cases of primary lymphoedema combined with recurrent cholestasis are reported. The cholestasis was of intrahepatic origin with defective excretion of conjugated bilirubin, bile salts and lipids. During the cholestasis malabsorption was demonstrated and the patients were jaundiced and complained of severe itching. From prepuberty the patients developed massive oedema in the legs, enamel defects and discoloration of the teeth. Severe pathology was demonstrated by lymphangiography in the lower extremities in three of the patients. In the non-icteric periods the biochemical parameters were normal, but the peripheral oedema persisted. Liver histology showed only slight degeneration of some of the parenchymal cells, but no progressive fibrosis. Hepatic radionuclide lymphography with colloidal ^{198}Au was performed in one patient. No lymphatic drainage was found suggestive of changes of the lymphatics in the liver. It is proposed that disturbed lymph flow of the liver is the main aetiological factor in the production of the recurrent intrahepatic cholestasis.

Several new syndromes of jaundice with increase in serum of conjugated bilirubin have been recognized in recent years. In Dubin-Johnson syndrome and Rotor syndrome there is probably a hereditary excretion defect of bilirubin from the liver cells. In 1959 Summerskill and Walshe (15) described the syndrome of benign recurrent intrahepatic obstructive jaundice. Subsequently patients with similar symptoms have been described (16, 17, 18).

In 1968 Aagenæs et al. (1) reported 16 cases of "hereditary recurrent intrahepatic cholestasis from birth". These patients developed oedema in the lower extremities in later childhood, and the oedema was the main problem for the patients as adults. Recent studies have shown that this oedema is primary lymphoedema with abnormalities of the lymphatic vessels (7).

Five of these patients are now adults, and a report of them is given with emphasis on the disease in adult life. A radionuclide lymphographic study of the liver in one patient is of special interest as it indicates disturbed lymph flow of the liver as an aetiological factor in the recurrent cholestasis.

CASE REPORTS

Case 1

A woman, now aged 26 years. Jaundice was noted on the 5th day of life. On the 8th day she had a life-threatening cord haemorrhage which was treated with vitamin K. From the age of 8 months she was troubled by itching which disturbed her sleep and kept her awake during the night. She remained jaundiced and complained of itching until the age of 6–7 years. In this period there was a growth retardation. The liver was enlarged and the spleen palpable. The stools were pale. Bile and urobilinogen were present in the urine. When the cholestatic period stopped, the "catch-up" growth started resulting in a final normal height (165 cm).

From the age of 10 years she developed oedema in the legs. In the beginning the oedema was present in the evening only. Later on, the oedema was present during the entire day though to a lesser extent in the morning. The oedema was the most severe problem for the patient. During the last few years she was treated with different diuretics with good effect.

At the age of 18 years she was again troubled by cholestasis which lasted for some months. During the following years her general health was good and all the biochemical parameters were repeatedly normal.

In September 1966 the stools became pale, the urine dark, and she was troubled by severe itching, nausea, and vomiting. She was admitted to the local hospital where she developed jaundice. Serum-bilirubin rose to 9.8 mg per 100 ml. From the biochemical findings it was concluded that the jaundice was due to intrahepatic

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Table I *Laboratory data*

Test	Case 1	Case 2	Case 3	Case 4	Normal values
Serum-bilirubin (mg/100 ml)	12.8	7.0	1 15	0 92	0.2 1.0
% unconjugated	35	30	82	58	
Serum bile acids (mg/100 ml)	2 3	3			< 1
Serum-cholesterol (mg/100 ml)					
Total	309	400	210	253	150-300
Free	120 (39 %)	160 (40 %)	72 (34 %)		45–75 (20–45 %)
Triglycerides (mg 100 ml)	186	335	17	32	50–150
Phospholipids (mg 100 ml)	273	349	184	202	180–300
Non-esterified fatty acids (mEq/l)	0.50	0.36	0 55		0 4–0.8
Total lipids (mg 100 ml)	1350	1470			400-1000
Lipoprotein electrophoresis	Hyper-β-lipoprotein Hypo-γ-lipoprotein	Hyper-β-lipoprotein Hypo-γ-lipoprotein	Normal	Normal	
Bromsulphalein retention test					
% retention after 45 min	33	42	3	15	< 5
120 min	32	44	3		
180 min	30	44	1		
Galactose tolerance test					
(T ½ value in min)	13	12	11	10	< 16
Oral glucose tolerance test	Normal	Normal			
Thymol turbidity (extinction units)	0.01–0 02 (range of 7 determinations)	0.01–0 02 (range of 5 determinations)	0 02		< 0 15
Prothrombin–proconvertin (Owren) ( )	89	80	72	78	80–120
Thrombotest (Owren) ( )	50	90	50	44	70–120
Quick-time	Normal	Normal		Normal	
Cephalin-time	Normal	Normal		Normal	
Serum-protein electrophoresis					
Total protein (g 100 ml)	5.2–6.4	6.3–7 1	5.9	7.6	6.5–8.0
Serum-albumin (g 100 ml)	2.2–4 0	2.1 3.2	3.2	3 7	3.3–4.9
α_1-globulin (g 100 ml)	0 1–0.4	0.3–0 6	0.2	0.3	0.2–0 4
α_2-globulin (g 100 ml)	0.5–0 8	0 8–1.0	0 8	1 1	0.4–0 8
β-globulin (g 100 ml)	0.8–0.9	1 1–1 4	0.6	0.9	0.6–1 0
Serum-γ-globulin (g 100 ml)	0.7–1.. (range of 7 determinations)	1 1 1 7 (range of 3 determinations)	1 1	1.6	0 9–1 7
Faecal fat (g 24 h)	49	15.5			6–8 g/24 h
Faecal nitrogen (g/24 h)	1 3	0.7			1.5 g 24 h
Vitamin A test (I U 100 ml plasma)	356	420			800
D-xylose-test (g excreted during 5 h)	8.6				> 4.5
Gastrointestinal protein loss					
$^{51}CrCl_3$ method	Normal	Normal			
Urine-bilirubin (Harrison spot test)	–			–	
Urine-urobilin (Schlesinger)	–			+	–

cholestasis. The patient did not respond to the administration of steroids. After one injection of vitamin K the thrombotest value (Owren) rose promptly. During the following 5 weeks she lost 6 kg in weight.

She was transferred to Medical Department A in October 1966. The skin and sclerae were markedly jaundiced. Numerous scratch marks on the trunk and extremities, moderate oedema in the legs, pronounced enamel defects, and discoloration of the teeth were noted. No hepatosplenomegaly palmar erythema, vascular spiders or naevi were found. There was loss of deep tendon reflexes. Her general health was relatively unimpaired. The results of some biochemical tests are shown in Table I and Fig. 1. (Bile acid determinations carried out by Dr J. Bremer, Department of Clinical Chemistry.)

Her jaundice disappeared gradually during the first months after she had left the hospital. Half year later she experienced another cholestatic period which lasted few months. In October 1969 she as again examined in Medical Department A. She was anicteric and all the biochemical parameters were normal. During corticosteroid treatment her oedema decreased in the left leg.

Case 2

A oman, now aged 25 years, as related to our first patient. Her mother had nine children. All her pregnancies and deliveries were uncomplicated. Three boys and one girl who were born before our patient died during the first year of life. According to the mother and the

family physician, these four children were all jaundiced. An elder sister (case 5) and second cousin (case 3) have been troubled by jaundice and oedema in the legs.

From the first week of life she was jaundiced. From the age of 2 to 5 years she was continuously troubled by itching of the skin all over the body. At the age of 9 years oedema developed in the legs, and she was considered to suffer from Milroy's disease. Otherwise she was symptom-free until August 1966 hen she was in the 7th month of her first pregnancy. At that time there as an onset of marked itching followed by jaundice and dark urine. Her oedema was more pronounced and she felt eak. Otherwise she had no symptoms.

On admission to our hospital in October 1966 she was markedly jaundiced with scratch marks on the feet and gluteal region and massive oedema in the legs. She had purple striae over the abdomen. The fundus uteri was palpable tw fingerbreadths below processus xiphoideus. Pronounced enamel defects and discoloration of the teeth, but no vascular spiders or palmar erythema ere noted. The deep tendon reflexes were absent. Albuminuria and pyuria were present, but blood urea and creatinine were normal. The results of some biochemical tests are shown in Table I and Fig. 1

Delivery occurred on November 14th, 1966, in the 38th week of pregnancy No obstetrical complications occurred. The female child was markedly jaundiced with increased conjugated and unconjugated bilirubin.

After delivery itching regressed and disappeared completely within one week. The liver was slightly enlarged and could just be palpated. Splenomegaly was not found.

During the following three eeks she lost 3.7 kg in weight and her oedema became markedly reduced. She felt completely well hen she left hospital on the 6th of December Her symptoms of cholestasis gradually subsided and disappeared during the following weeks. She had, however, new cholestatic period in 1967 during the last trimester of her second pregnancy and delivered healthy anicteric female child. I 1969 she was again icteric during the last trimester of her third pregnancy

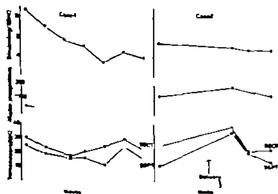


Fig 1 Course of serum bilirubin, alkaline phosphatases and transaminases in case 1 and case 2 during their stay in Medical Department A. Normal values: alkaline phosphatases 10–45 U/1, SGOT 5–20 U 1, SGPT 2–17 U/1.

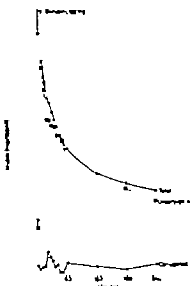


Fig Plasma disappearance of unconjugated and conjugated bilirubin following intravenous injection of 102 mg bilirubin in case 3 during non-icteric period.

Case 3

A man, now aged 27 years. During the first year f life he was icteric and troubled by itching, and the stools were pale and the urine dark. At the age of 3 and 5 years he was again troubled by cholestasis hich lasted for some months.

From the age of 9 he was troubled by increasing oedema of the legs from the feet to the thighs and genitalia. Periodically he also had oedema in the hands. His oedema varied in intensity and this variation was paralleled by change in body weight between 74 and 85 kg.

On admission to Medical Department A in January 1969 he had massive oedema of the feet, calves, thighs, and scrotum. At the distal part of the legs his oedema was prominent like tumours. A slight yellow discoloration of the teeth was noted. No hepatosplenomegaly palmar erythema, vascular spiders or ascites ere found. The results of some biochemical parameters are shown in Table I.

Plasma osmolarity was normal (304 milliosmols/kg) Aldosterone excretion was 14 μg /24 h (slightly elevated) A slight elevation was found of the red cell volume (121%) and plasma volume (124%).

Cholecystography showed poor concentration of contrast in the gall bladder, hich had normal form and size. Roentgen examination f oesophagus, stomach, and duodenum revealed no abnormality Angiography of the inferior caval vein showed no pathology and the venous pressure was normal (2–4 mm Hg)

The plasma disappearance curve of conjugated and unconjugated bilirubin was examined following intravenous injection of bilirubin by the method described by Billing et al. (4). The bilirubin excretion was normal in the non-icteric period (Fig. 2).



Fig 3 Lymphangiograms of case 1 ( ) Filling of numerous collateral pathways, partially in the interstitial ue. (*b*) Delayed clearing of the contrast demonstrated on film taken 24 hours after injection of contrast. (*c*) Only one opacified draining lymphatic vessel in the thigh. Film taken during injection of contrast.

Case 4

A boy now aged 18 years, brother of case 1. During the first year of his life he had jaundice, dark urine, and pale stools. From the age of 7 to 11 years he had 3–4 attacks of cholestasis which lasted about tw months. From the age of 8–9 he had oedema in the legs. During the last year he has been treated with diuretics w th good effect.

On admission to Medical Department A in June 1968 he had slight oedema of the feet and calves. The scrotum was enlarged due to bilateral hydrocele. Also the testes ere enlarged. A slight yellow discoloration of the teeth was noted. The illary hair was sparse. There was no hepatosplenomegaly, palmar erythema, vascular spiders or ascites. The result of some biochemical parameters are show in Tab.e 1.

Case 5

A woman, now ged 34 years, sister of case 2. She had her first cholestatic period shortly after birth, and was varyingly jaundiced until about 6–7 years of ge. She has experienced five cholestatic periods. Several times she has undergone plastic surgery for "elephantiasis" in the legs. T ice the cholestatic periods with jaundice have been provoked by the operations. She was amined January 1967 in a non-icteric period. Thick, oedematous feet and enamel defect and discoloration of the teeth were noted. Otherwise she had no signs of disease and felt well. The biochemical parameters ere normal, including the bromsulphalein retention test.

RESULTS

Hepatic histology

A needle biopsy was taken from cases 1 – 3 and 4. From cases 1 and – the biopsy wa taken during cholestatic periods. Cases 3 and 4 were investigated in non-icteric periods. Microscopically the four liver biopsies showed almost the same picture. The architecture was preserved. In some areas there was a pseudotubular arrangement of the parenchymal cells. There was a slight degeneration of some of the parenchymal cells and a slight increase in the amount of connect ve tissue. Some binucleated, multinucleated or giant cell were seen among the parenchymal cells. In cases 1 2 and 3 the parenchymal cells and prominent

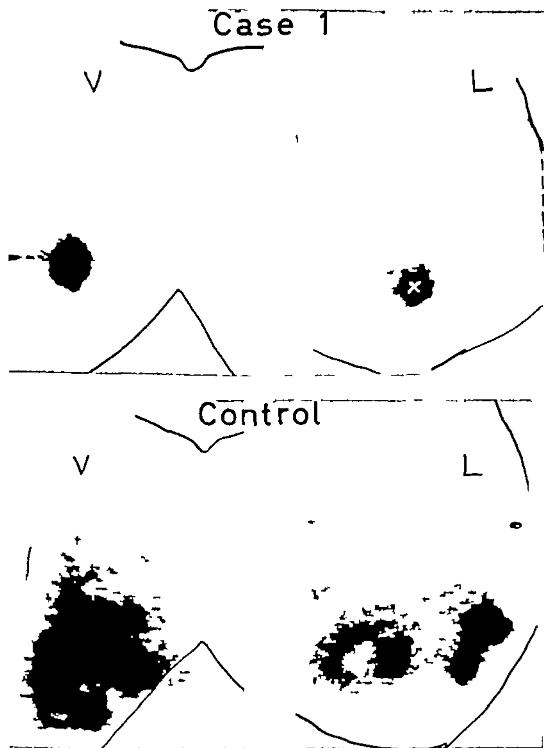


Fig. 4 Photoscintigrams of radiocolloid distribution 24 hours after transcutaneous intracapsular hepatic injection of 70 μCi ^{198}Au with particle size 30–50 Å. Antero-posterior (V) and right lateral (L) views of case 1 and normal control are shown. Site of injection is marked by arrow or x.

Kupffer cells contained an increased amount of a yellow-brown bile pigment. In cases 1 and 2 bile canaliculi were dilated and contained many bile casts. (Histological examinations carried out by Dr S Refsum d.y., Institutt for Generell og Eksperimentell Patologi, University of Oslo.)

Electron microscopy is under preparation.

Lymphangiography

Lymphangiography (9 10 11) in the lower extremities was performed in three of the five patients, all of them being in a non-icteric period. Case 5 had been operated upon by plastic surgery and was therefore unfit for lymphangiography and case 2 rejected the examination. The lymph angiograms were abnormal in all the three patients examined, with hypoplasia of the lymphatics, collateral filling with dermal backflow and retarded emptying of contrast from the lymphatics (Fig. 3).

Hepatic radionuclide lymphography

Hepatic radionuclide lymphography was performed in case 1 in October 1969 in a non-icteric period with normal biochemical findings. This lymphography was carried out by the trans- and intracapsular injection of colloidal n, described by Magnenat and Delaloye (12,) The injection was made in the mid-axillary ne in the 9th intercostal space (Fig. 4) No lymphatic drainage was observed during the first 7_ hours, suggesting severe changes of the lymphatics.

DISCUSSION

Lymphangiography of a lower extremity in cases 1 3 and 4 showed severe changes of the lymphatics. The oedema was regarded as primary lymphoedema. Lymphangiography carried out in a few affected children in the family revealed the same pathological changes of the lymphatics (2). The clinical picture in our cases was identical to hereditary idiopathic lymphoedema which often starts in childhood or adolescence and is then called lymphoedema praecox.

The oedema was most pronounced in the legs, but periodically two patients had distinct oedema also in the hands. One of the patients (case _) had transient slight albuminuria, but no cylindruria or other signs of renal disease There was no evidence of cardiac disease in the five patients.

The extent and variation of the oedema were probably dependent on secondary factors. Hypoalbuminaemia was demonstrated (Table I) in some patients and might have been an important factor Massive oedema was noted, however in periods when the serum protein values were normal.

The evidence of primary lymphoedema in the lower extremities in all cases examined might suggest lymph vessel abnormality in the liver as a possible cause of cholestasis. Our knowledge of the anatomy of the liver lymphatics, and even more of the physiology of the vessels, is poor There is an intimate relationship between the bile ductuli and the lymph capillaries (5). By obstruction of the bile duct, bile constituents increase promptly in the liver lymph. On the other hand we know nothing about the effect on the bile excretion of lymph stasis and lymph hypoplasia. Hungarian authors (3 6) have studied the effect of artificial obstruction of the liver lymphatics in cats. Histologically this obstruction was followed by a pronounced dilatation of the Disse space (3) and histochemically a decrease in different enzyme systems was found (6). It does not seem improbable that a lymph stasis may cause biliary obstruction, either by damage to enzyme systems necessary for the bile excretion or by compression of the bile ductuli by distended lymphatics. So far however we have no histological or experimental evidence for any of these hypotheses.

The finding of lack of drainage of colloidal radiogold after intracapsular injection in one of our patients indicates abnormal lymph function. Retention of the radiocolloid in the liver capsule has been described in liver cirrhosis (1_ 13). We have no experience with radionuclide lymphography in cirrhosis, and it is impossible to say whether the registered lack of drainage in case 1 is due to primary lymphatic insufficiency or secondary cirrhosis. The fact that there was only a slight increase in the amount of connective tissue and no evidence of regeneration nodules points to the first alternative as the more plausible

The patients suffered from recurrent intrahepatic cholestasis. The normalization of the bile excretion in childhood points to some sort of

compensation. The two known causes of recurrent cholestatic periods are anaesthesia and pregnancy. Anaesthesia usually slows the bile flow and pregnancy (latest months) may act by a mechanical slowing down of the bile flow. It therefore seems possible that a bile flow which is just sufficient may lead to cholestasis by a moderate slowing down of the flow.

The absence of histological evidence of severe chronic liver damage and the recovery from each of the many episodes of jaundice show that the disorder is relatively benign. The clinical and histological characteristics differ from the syndromes with hereditary conjugated hyperbilirubinaemia, Dubin-Johnson syndrome and Rotor syndrome. In these syndromes there is an excretion defect of bilirubin, while our patients have an excretion defect of bile acids and lipids, as well as of bilirubin.

The malabsorption is due to the lack of bile in the bowel during cholestasis. The enamel defects and discoloration of the teeth are probably results of the malabsorption and the hyperbilirubinaemia.

Our patients correspond in many ways to the cases described by Summerskill and Walshe in 1959 (15) and later by Tygstrup (17), de Groote et al. (7) Schapiro and Isselbacher (14), and Summerskill (16). They differ however from the Summerskill type of cholestasis in their development of oedema in prepuberty, the presence of giant cells in liver biopsies, a clear genetic factor, the start of the first cholestatic period at birth or in early infancy and the constant hyperlipaemia in the cholestatic periods.

In 1966 Juberg et al. (8) reported four siblings with intrahepatic cholestasis from infancy characterized by pruritus and slight hyperbilirubinaemia. These children developed progressive liver fibrosis and differ therefore from our cases.

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MICROSCOPICAL HAEMATURIA AFTER INJECTION OF HEPARIN

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Abstract. The number of red cells per field of vision *in fresh urinary sediment was counted before and two* hours after intravenous injection of heparin, 200 units per kg body weight, in 31 healthy subjects and 370 patients with diseases of the kidneys or urinary tract. After the injection a distinct increase of the red-cell count in the urinary sediment was noted, commonly (about 50%) in patients with chronic glomerulonephritis or nephrolithiasis, less commonly in patients with collagen diseases, cysts, sarcoidosis or tumours, and still less commonly in patients with chronic pyelonephritis, lower urinary tract infection, proteinuria of obscure origin, or essential hypertension. In healthy subjects heparin injection was followed only by a slight increase of the red-cell count in the urine. Because of its low specificity the heparin test is suitable only as a screening test, most appropriately in the investigation of ambulatory patients who are found to have proteinuria or hypertension without haematuria, as a means of detecting evidence of glomerular disease. Because of the great variations in the excretion of red cells in patients with haematuria and of the technical errors in the counting of red cells, particularly in urinary sediment, the test is less suitable for investigation of patients with microscopical haematuria. However, in these cases the heparin test seems to be of value in assessing whether a *glomerular disease is decreasing or increasing in intensity.* The number of red blood corpuscles in the urine should be counted by using uncentrifuged urine.

Gross haematuria is seldom seen after injection of heparin. Microscopical haematuria, on the other hand, may occur after heparin injection (1). We noted a distinct increase of the number of red cells in urinary sediment in more than 50% of the patients with chronic glomerulonephritis or nephrolithiasis, in 10 to 50% of the patients with collagen diseases, sarcoidosis, renal cysts or renal tumours, and in 4 to 9% of the patients with chronic pyelonephritis, lower urinary tract infection or benign proteinuria. Microscopical haematuria following heparin injection was not seen in patients with essential hypertension or in healthy subjects without urinary tract disorders. We proposed that the different reaction to heparin might be used clinically and named the test the "heparin sediment" test. It may possibly be used as a screening test, particularly for proteinuria, hypertension or microscopical haematuria, as a means of detecting cases of suspected glomerular disease and renal or urinary stone. Grading of the intensity of a glomerular disease might also be within the range of usefulness of the test. We have now collected a larger series and thus gained further experience with the test.

MATERIAL AND METHODS

The patient was not prepared. Examination within the first three days after X-ray or surgery of the urinary tract was avoided. The test was performed at midday. Fresh urine sediment was examined immediately before and two hours after intravenous injection of heparin, 200 units per kg body weight. The urine was centrifuged for five min at 4000 r.p.m. The number of red cells per field of vision (magnification 400 times, the mean of at least five fields) was counted.

Thirty-one healthy subjects and 370 patients with renal or urinary tract diseases were examined on 470 occasions. In the patient group the diagnoses in cases of manifest renal diseases were verified by kidney biopsy in more than 80%. Cysts or tumours of the kidney were demonstrated by selective renal angiography. Kidney biopsy was restrictively performed in patients with essential hypertension and in those with benign juvenile proteinuria. The patients with sarcoidosis or collagen diseases had impaired renal function or proteinuria as evidence of renal involvement.

RESULTS

Table I shows the total series. An increase in the degree of haematuria, here defined as a rise by at least one class in the table, occurred in

variations in biological excretion of red cells plus technical errors.

In our experience however the heparin test is of some clinical value in the following situations.

1 Obscure cases of proteinuria without haematuria in ambulant patients. These include the large categories of patients with benign juvenile proteinuria and of persistent proteinuria after pregnancy in whom it is preferable to postpone renal biopsy for a few years. The heparin test might help to sort out some patients of these categories who would need further investigation or check-ups.

2 Middle-aged patients with essential hypertension. Among these would be a small number of cases of chronic glomerulonephritis, some of which could possibly be revealed by means of the heparin test.

3 To assess whether a glomerular disease is changing for the better or the worse. The heparin test is clearly superior to following the AS titre (pathological test in 70 cases of chronic glomerulonephritis, or 65% as against 0% by the AS).

Side-effects in the form of bleedings from other organs did not occur Macroscopical haematuria developed in one patient with renal stone.

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EFFECTS OF PROLONGED FAST ON LIPOPROTEIN LIPASE ACTIVITY ELUTED FROM HUMAN ADIPOSE TISSUE

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Abstract. Lipoprotein lipase activity of subcutaneous adipose tissue from nine obese subjects has been measured before and during total fast up to 13 days. The activity decreased during the first eight days but then there was a tendency of recovery. FFA in serum remained increased throughout the fast. No significant changes occurred in serum levels of triglycerides and cholesterol.

It has been shown by a number of authors that the lipoprotein lipase activity of the rat adipose tissue after a fast of 24–48 hours falls to less than 1/5th of the value obtained in the fed animals. On re-feeding the activity rises rapidly to a level which might be equal to or higher than the initial findings (3, 11, 14, 17, 18).

The blood levels of lipoprotein lipase activity after heparin injection have, however, been reported not to fall much during fasting (13, 18).

Information in man is scanty—but Kuo et al. (12) reported that the response after the heparin injection test standardized by Fredrickson et al. (8) decreased on low caloric intake and increased after high caloric intake. In human adipose tissue Dempott and Kerpel (5) noted an increase of lipoprotein lipase activity after a fat-rich meal in four out of five tested subjects.

The conditions for elution and determination of lipoprotein lipase activity in human adipose tissue have been reported earlier from our laboratory (15). A significant inverse relationship between the lipase activity and the serum triglyceride level up to about 300 mg per 100 ml was also shown (15).

In the present report we have studied the influence of prolonged fasting on the lipoprotein lipase activity.

MATERIAL

The clinical material consisted of nine grossly obese subjects who volunteered to submit themselves to a 10–13 days period of total starvation for the purpose of the study. Some of the characteristics of these subjects at the outset of the study are given in Table I.

Before the experimental period the patients were on the ordinary hospital diet—without close supervision. During the fast they were allowed tea and water ad libitum. They had freedom of movement. Although it was repeatedly stressed that deviation from the agreed total abstinence of food might jeopardize the above study, it is probable that some breaks in fasting may have occurred for one of the patients (no. 7).

One patient was on hydralazine throughout the study. All had vitamins. Otherwise drugs had been excluded.

METHODS

Percutaneous needle biopsy specimens were obtained as described earlier (10, 15). However, in comparison with earlier descriptions (15, 16) the following changes were made in the elution of the enzyme from the adipose tissue and in the enzyme assay system.

1. pH in the elution medium was adjusted to 7.7 with Tris buffer at a 0.025 M concentration.

2. Elution of biopsy specimens was done under identical conditions in four consecutive periods of 30 min. This gives, as described in a previous paper (15), a stepwise fall in lipoprotein lipase activity with usually very low activity in the fourth eluate. The results of these procedures have been given as the sum of activities in the four eluates.

3. In the lipase assay system, as described earlier (15), the component of 0.1 ml 15% albumin is present in a solution of 0.30 M ammonium sulphate before pH adjustment, which by error was not stated in the previous paper.

If enough material was available, samples of the biopsy were eluted in duplicate. The assay of the eluates for lipoprotein lipase activity was performed as given in the previous paper (15) based on triplicate measurements.

Table I *Clinical and laboratory features*

Subject no., sex and diagnosis	Age	Height (cm)	Weight (kg) Highest noted	Before fast	After fast	Before fast in serum Aceto-acetate (mg 100 ml)	Triglycerides (mg 100 ml)	Cholesterol (mg/100 ml)	Free fatty acid (μEq l)	Glucose (mg 100 ml)
1 ♀ Obesity hypertension	56	165	143	121	113	—	109	345	660	64
2 ♀ Obesity lumbago	45	170	97	96	89	0.93	700	47	600	60
3 ♀ Obesity hypertension	53	169	132	132	120	—	87	202	914	95
4 ♀ Obesity	65	158	149	120	110	1 17	129	210	938	90
5 ♀ Obesity	44	170	98	98	91	—	117	177	573	80
6 ♂ Obesity hypertension	24	167	114	110	105	—	305	270	702	75
7 ♀ Obesity	50	156	110	88	83	1 45	168	276	964	73
8 ♀ Obesity	7	157	73	68	61	0 88	125	228	908	71
9 ♀ Obesity	66	170	138	114	104	3.51	105	176	1777	100

f free fatty acids (FFA) according to modification 1) of the method of Duncombe (7).

The activity has been expressed as μEq FFA released/ min g wet eight f adipose tissue.

DNA analyses of the adipose tissue were performed according to the method of Webb et al. (22).

The biopsies were taken in the morning after t least 1_ hours fasting. At the same time morning blood sample as taken. Analyses ere made in duplicate. Cholesterol was measured according to the Cramér and Isaksson (4) modification of the Thorell (19) procedure, triglycerides according to Carlson (2). FFA in serum according to Dole (6), aceto-acetate according to Walker (21), and glucose according to the glucose xidase method of de Verdier et al. (20).

RESULTS

The variations of the lipoprotein lipase activity during the fast as measured in the first eluate for each individual are shown in Fig. 1 *a* and *b*. The purpose of the partition of the figures into *a* and *b* with data from three subjects in one and six in the other was simply to make it easier to follow the individual curves. However the three most hypertriglyceridemic subjects appear in Fig. 1 *b*.

It is evident from the curves that the activity of most subjects falls during the first week of the fast. The results obtained after more than eight days of fasting, however seem to show a recovery in activity. Therefore we have divided the time of the experiment into one basal period and two periods of early (1–8 days) and late (9–13 days) fast respectively.

For each period means of lipoprotein lipase activities for each subject have been calculated (Table II). The differences in activity between the basal period and the periods of early and late fast as well as between the two fasting periods were also calculated, and a summary of these results and also those of FFA analyses in blood appears in Table III. There is a significant ($p < 0.01$) fall in lipoprotein lipase activity during the first eight days of fast. It will also be seen that there is a significant ($p < 0.05$) recovery of activity as measured in the sum of four eluates from the period of early fast to that of late fast.

In contrast to the changes of lipoprotein lipase activity serum FFA remains significantly increased during both f sting periods as compared with the basal.

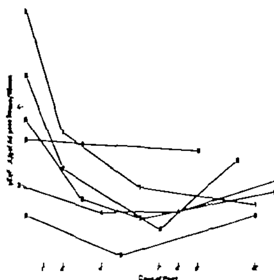
DNA analyses of the adipose tissue biopsies were made on all samples which had earlier been quantitized by means of wet weight. The relation between wet weight and DNA for the nine subjects was in the range of 9700 ± 2900 (mean ± S.D.) and did not change significantly during the fasting period. Of the remaining serum analyses during the fast aceto-acetate was analyzed in five subjects. With the exception of one subject (no. 9) who had an amazingly high basal value, the others showed an increase to, on an average, 6–12 mg per 100 ml. Cholesterol, triglyceride and glucose were analyzed for most patients in each period and did not show any significant changes.

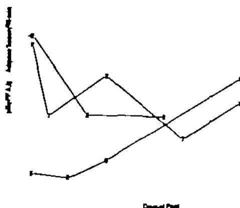
The figures for weight reduction have been given in Table I. In three subjects we followed the FFA release from samples of adipose tissue in Ringer's solution adjusted to pH 6.8 with Tris buffer without any addition of exogenous substrate but with albumin added as a FFA acceptor. In comparison with the lipoprotein lipase activities described above, the FFA release at pH 6.8 without added substrate was low (~0.8 μEq/g tissue/45 min) and did not increase during the fast.

DISCUSSION

The finding of a decrease in lipoprotein lipase activity in human adipose tissue during fast is in agreement with experiments in animals, predominantly the rat (3, 11, 14, 17, 18). Differences between species and size might explain why it took a longer time to induce changes of lipoprotein lipase activity in man than in rats and also why there was a greater variability in the results. Thus, for example, the subject with the highest triglyceride value in serum started from the lowest initial lipoprotein lipase activity but then increased his activity to a final comparatively high activity. On the other hand the subject with the highest initial activity was the only one to decrease her activity throughout the fast. Differences between species might also explain why the final recovery of activity has, to our knowledge, not been reported earlier. It may be of interest to note that the variance of activity seems to be lowest during the final period.

Hypothetically the primary decrease in lipoprotein lipase activity might be explained by the general state of protein catabolism during fast





b

Fig. 1 a and b. Lipoprotein lipase activity of subcutaneous adipose tissue measured in the first eluate during total fast. (a) Six subjects with normal values of triglycerides in serum. (b) Three subjects with slight to moderate hypertriglyceridemia.

and or by decreased serum insulin and glucose levels.

That the measured lipolytic activity during nonfasting conditions really is due to lipoprotein lipase is highly probable (16), while this statement might not be as valid under fasting conditions. However with the medium for incubation

Table II *Lipoprotein lipase activities before and during total fast*

Number of observations in each period on which the values are based is given within brackets

Subject no	First eluate			Sum of four eluates		
	Before f st	Day 1–8 of fast	Day 9–13 of fast	Before fast	Day 1–8 of f st	Day 9 13 of fast
1	6.45 (?)	2.67 (2)	1.50 (1)	8.7? (?)	4 15 (2)	3.50 (1)
2	4 00 (1)	1 95 (2)	—	8.51 (1)	3 13 (2)	—
3	3.17 (2)	2.96 (1)	2.87 (1)	4 18 (2)	4.83 (1)	5.58 (1)
4	1 96 (2)	1.32 (2)	2.10 (1)	3.76 (?)	1 73 (1)	3 71 (1)
5	3.69 (2)	1 38 (2)	1.82 (1)	4.53 (2)	2 03 (?)	3 83 (1)
6	0.42 (2)	0.54 (2)	2.91 (1)	2.01 (2)	.36 (2)	6.58 (1)
7	3.80 (?)	2 09 (3)	2.28 (1)	7.33 (2)	4 06 (3)	4 13 (1)
8	4 78 (2)	1 69 (2)	2.63 (1)	8 75 (2)	5 44 (2)	6 10 (1)
9	1.21 (?)	0.15 (1)	1.20 (1)	2.76 (2)	1.33 (1)	4.8 (1)
Mean value	3.28	1 64	.16	5 62	3.23	4 82
± S D	±1.85	0.92	±0 63	±2.71	±1 46	±1 18

Table III *Changes in lipoprotein lipase activities of adipose tissue and FFA in serum from the basal period (B) to the periods of total fast and from the first (I) to the second (II) period of fast*

	Means of differences ± standard deviation		
	B I (9)	B→II (= 8)	I→II (n = 8)
First eluate	−1.65 + 1 31 p 0.01	−1 02 + 2.17	0.56 ± 1.02
Sum of 4 eluates	−2.39 + 2.03 p 0 01	0 48 3 17	1.54 ± 1.67 p 0 05
ΣA	401 228 p 0.005	451 + 365 p < 0 01	—

used by us, we did not find any increase in FFA release from the adipose tissue at pH 6 8 in contrast to the findings of Goldrick and Hirsch (9) with their slightly different technique. This does not exclude an increased leakage of the "hormone-sensitive lipase to the elution medium but the assay conditions for the lipoprotein lipase activity do not favour the other lipase

ACKNOWLEDGEMENT

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ACHILLES REFLEX TIME AS A MEASURE OF THYROID FUNCTION

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Abstract. An analysis has been made of the method for determination of the Achilles reflex time (called reflex time). Different sources f error are reported. A high reliability of the test was obtained with the described method. The reflex time and basal metabolic rate (BMR) of 136 patients, 25 hyperthyroids, 98 euthyroids and 13 hypothyroids, are measured, and to less extent the PBI and ^{131}I uptake. About one third of the patients had earlier undergone treatment of the thyroid gland, all except few with ^{131}I. Reflex time showed fairly high validity as test of thyroid function. With normal values for the thyroid function tests used at the Hospital of Umeå there were small differences between the total number of true diagnoses based on reflex time, BMR, PBI and 24-hour uptake of ^{131}I (T_{24}). The normal range for reflex time seemed to be little affected by extra-thyroid factors or earlier treatment of the thyroid. The reflex time is therefore suitable for evaluating the state of patient hose thyroid gland has been treated and for checking the effect of treatment. As regards diagnosis of thyroid function, combination of reflex time and BMR gave better discrimination in the examined group of patients than either test used separately. The diagnostic accuracy as about 90 in euthyroids and about 80% in hyperthyroids and hypothyroids. N other combination of tw of the thyroid function tests gave better result than that of reflex time plus BMR.

A change in the velocity of the tendon reflexes in hypothyroidism was observed in 1884 by Ord (20), but the first attempt to produce a graphic record of the reflex was not made until 1924 by Chaney (4). He observed that the full response to a tap on the Achilles tendon was of longer duration in patients with myxedema than in normal control subjects or in euthyroid subjects with low basal metabolic rates associated with certain other conditions. In two cases with myxedema, described by Eckerstrom in 1936 and 1938 (7 8), the prolongation of the Achilles reflex had been helpful in diagnosis. Since then various types of apparatus have been designed for recording the Achilles reflex (11 13 15 19 4 27). Two of the most widely used seem to be the Photomotograph (11), which records the Achilles reflex by means of a photoelectric device and the Kinemometer (15) which uses an electromagnetic arrangement.

Many authors have found a significant correlation between Achilles reflex time and thyroid function (2, 3 5 9 13 15 18, 24). In spite of this the test has not been generally employed. Some authors have doubted the value of the Achilles reflex time as a valid test of thyroid function (22, 23 30).

The inconsistency in the results of different authors makes it urgent to analyze the methodology and compare the Achilles reflex time with other tests of thyroid function.

MATERIAL

Normal values for the Achilles reflex time (hereafter called reflex time) are obtained from 29 control subjects with no clinical signs of thyroid disturbance. There were 21 females and eight males, ranging in age from 25 to 61, ith mean age 41

Reflex time was measured in 61 in-patients referred from the Department of Radiotherapy and in 73 in-patients referred from the Department of Internal M dicine to the Department of Clinical Physiology of the Hospital of Umeå for measurement of the basal metabolic rate (BMR) because of suspected thyroid function disturbance. The ages ranged from 18 to 79 with mean age 50. One hundred were females and 36 males.

In the Hospital of Umeå almost all patients suspected of having disturbance of thyroid function are examined by measurement of the BMR. During the first half f 1965 160 patients were selected for determination of reflex time. Nine patients were excluded because of defective examination of reflex time, and 19 because of missing medical journals or of uncertain or missing diagnoses of thyroid function. Four patients from the Department of Radiotherapy later examined and diagnosed as hypothyroids, were also included in the material.

Table I. *Patients from the Departments of Internal Medicine and Radiotherapy examined with BMR only and with reflex time + BMR*

Distribution of total number of patients, mean percentage of BMR, percentage of hyperthyreosis and hypothyreosis

	BMR-examined patients				Patients examined with reflex time + BMR			
			Diagnosis (% of total)				Diagnosis (% of total)	
Department	Total	Mean BMR (%)	Hyper-thyreosis	Hypo-thyreosis	Total	Mean BMR (%)	Hyper-thyreosis	Hypo-thyreosis
Internal Medicine	337	6.3	11.9	7.1	73	4.1	11	9.6
Radiotherapy	89	17.2	31	2.2	63	13.8	27	10

Patients from the Department of Radiotherapy were examined in the first place. Otherwise the selection was random. Thus, although the selection was not fully randomized, the selected group did not seem to differ from the whole group of BMR patients from the two Departments (Table I).

The assessment of the patients' thyroid state—"the final diagnosis"—was made by clinicians in the various wards at the time of the actual examination on the basis of clinical and laboratory data including BMR, protein-bound iodine (PBI) and radioiodine tests—but reflex time was excluded. The validity of the diagnosis was later confirmed after following the course of the patients' illness. Twenty-five of the patients were hyperthyroid, 13 hypothyroid, and the remaining 98 euthyroid (Table II).

During the four weeks preceding the examination, one ... and ... euthyroid patients had received treatment. None had actual substitution therapy with thyroid medication. Forty-four subjects had received ^{131}I treatment; 31 of them were considered euthyroid, ten hyperthyroid and three hypothyroid at the time of the reflex time examination. Since the last treatment with ^{131}I an average of 1 months had elapsed for the patients who were considered euthyroid at the time of reflex time examination (range 1.5–48), three months (range ...5) for the hyperthyroid patients, and four months (range 3–5) for the hypothyroid patients. Patients who had not received any treatment before the examination will hereafter be referred to as untreated, and those who had received any treatment of the thyroid gland as treated.

Table II. *Number of cases with breakdown by diagnoses and different thyroid function tests*

Basal metabolic rate (BMR), reflex time, protein-bound iodine (PBI), ^{131}iodine uptake in the thyroid after 2 h (T_2), after 24 h (T_{24}), and urine excretion over a period of 24 h (U_{24})

	No.	BMR	Reflex time	PBI	T_2	T_{24}	U_{24}
Euthyroids	98	98	98	53	45	62	61
Hyperthyroids	25	25	25	13	19	4	24
Hypothyroids	13	13	13	9	6	9	9
Total	136	136	136	75	70	95	94

METHODS

Measurement of Reflex Time

Apparatus

The Kinemometer according to Lawson (15) consists of three elements: a horseshoe magnet taped to the patient's bed, two coils wound on two identical soft-iron, L-shaped cores functioning as detectors of the movement of the magnet, and a standard one-channel electrocardiograph. The inductive current is generated by the movement of the magnet and is recorded as a tracing on the electrocardiograph, indicating the velocity and direction of movement. The paper velocity is 50

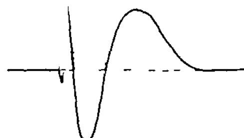


Fig. 1. The normal reflexogram obtained with kinemometer. Point *S* corresponds to the tap on the Achilles tendon. Phase *S-C* includes oscillations provoked by the tap on the Achilles tendon and the propagation time of the reflex impulse to and from the spinal cord. Phases *C-D* and *D-F* correspond to the contraction and relaxation respectively of the calf muscles. Points *M* and *M* correspond to the highest velocity of contraction and relaxation, respectively. Interval from *S* to *D* used as measure of reflex time.

mm/sec, in the later part of the investigation 100 mm/sec.

As the movements of contraction and relaxation are in opposite directions, the generated electromotive force is also in different directions in the two phases. The phase of contraction can therefore be separated from the phase of relaxation.

Reflexogram

The recorded tracing (Fig. 1) is composed of three phases: the initial oscillations—phase *S-C* in Fig. 1—are provoked by the tap on the Achilles tendon. These fairly high-frequency oscillations are succeeded by a slower movement of the heel-magnet when the reflex stimulus has been conducted to and from the spinal cord and produced a contraction of the calf muscles. M and M_1 correspond to the highest velocity of the contraction and of the relaxation respectively. *D-F* is the relaxation phase. The interval from *S* (the response to the reflex stimulus) to *D* (the end of the contraction phase) has been used as a measure of reflex time.

Technique of measurement

All measurements were taken with the patient kneeling on a chair. They were taken at various times of the day and were not preceded by any standardized conditions. Repeated reflexes were elicited until eight technically satisfactory tracings were obtained, four from the right and four from the left side. In about 10% of the examined subjects an Achilles reflex was elicited from neither leg nor from one leg only (in the latter case the mean value of eight recordings from one side represented the patient' reflex time).

The dynamic properties of the apparatus

Phase distortion of the recording system was determined by allowing the magnet to be moved according to a sine wave function with various frequencies. Assuming the *C-D* interval to correspond to half a cycle and the *S-C* interval to a whole cycle, the error in the *S-D* interval due to phase distortion could be calculated (Fig. 2). The

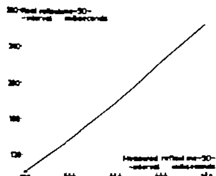


Fig. 2. Relation between the real or true *S-D* interval obtained from the measured *S-D* interval after corrections for phase differences in the various phases of the reflexogram and the measured *S-D*-interval.

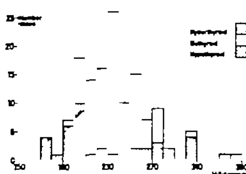


Fig. 3. Diagram showing the number of hyperthyroids, euthyroids and hypothyroids among the examined patients in relation to measured reflex time.

reflex time values in this report are not corrected for phase distortion.

Abnormal direction of movement of magnets in relation to induction coils can explain only a small fraction of the total number of distortions of the tracings. Most of them seem to be caused by secondary vibrations in the foot/leg in conjunction with the tap on the heel. It is therefore important to support the distal part of the leg so that it remains stable.

Methodological errors

The *S-C* interval (Fig. 1), corresponding to the interval from the stimulus of the Achilles reflex—the tap on the Achilles tendon—to the end of the contraction, was used in this investigation, as in other studies with the Kinemometer, as an expression of thyroid function. It is expressed in milliseconds and given as the mean value of eight measurements. The standard deviation of a single determination was 12.0 msec, corresponding to a coefficient of variation of 5.1%. The non-systematic errors decrease with the number of measurements, and after eight measurements the standard deviation of the mean was 4.2 msec, corresponding to a coefficient of variation of 1.8%.

A systematic deviation—here termed asymmetry—often existed between the reflex time of the two sides, and therefore as a rule four tracings were made from the right and four from the left side. In thirty consecutive patients a difference in *S-D*-values was obtained between the two sides, changing at random, an asymmetry that was statistically highly significant ($p < 0.001$).

Other Laboratory Tests of Thyroid Function

Basal metabolic rate (BMR) was determined in the Department of Clinical Physiology by means of a double test using a Krogh spirometer. Measurements of protein-bound iodine (PBI) were made in the Department of Clinical Chemistry according to a method described by Jacobsson and Widström (12). The measurements of ^{131}I uptake in the thyroid gland after 2 hours (T_2) and after 24 hours (T_{24}), and of excretion in the urine over a period of 24 hours (U_{24}), were made in the Department of Radiotherapy. All patients were not subjected

Table III *Normal range for different thyroid function tests used in Umeå Hospital*

Mea nd limit for +1 S D f om mean of values for same test for all examined euthyroids (both untreated and tre ted) on the one hand nd for untreated (see Material) or treated (see M terial) on the other

	Normal range	Euthy oid group	Tre ted euthyroid	Untreated euthyroid
Reflex time msec	214 232	237 216–259 (−98)	238 216–260 (41)	236 214–258 (−57)
BMR	10 15	5 7 16 (98)	5 −7 16 (41)	+5 −8– 18 (−57)
PBI	3 4 7 6	5 2 2.9–7.5 (53)	4 7 2.1 7.3 (17)	5.5 3.3–7 7 (36)
T_{3r}	24 31	36 23–50 (62)	34 17 50 (n−36)	39 29 50 (n−26)

to ev ry investigation (Table II) Reflex time was compared with BMR, protein-bound iodine and T_{3r} f the radioiodine tracer test as discriminator of thyroid function. The 4-hour uptake of radioiodine was selected, as in the international literature it is the most used variable of the radioiodine tracer tests. For diagnosis of the thyroid state all tests were taken into consideration by the clinician, including the time pattern of ¹³¹I uptake.

The normal range for BMR, used at the Department of Clinical Physiology emanates from regression equa tions given by Harris and Benedict (11) Values from −10 nd less nd from +15% and more were considered to fall outside the normal limits 95% of group healthy blood donors ere included within the range normal alues for PBI used t the Department of al Chemistry and ranged from 3 5–7 5%

normal ange for T_{3r} used at the Department Radiotheraphy as 25–30% and corresponds to about ±1 S D f om mean for healthy subjects (6)

RESULTS

The distribution of hyperthyroids, euthyroids and hypothyroids among the examined patients in relation to reflex time is shown in Fig. 3 There is a c rtain overlapping of the reflex time values of hyperthyroids and euthyroids, and of hypothyroids and euthyroids The three groups are distingui hed from each other by different mean values that are statistically highly significant. The reflex time for 9 healthy control subjects, 233 ± 18 msec (mean S D), coincided closely with the euthyroids (Table III)

The range of 1 S.D from the mean in the values of *BMR, PBI* and T_{3r} *for the examined* euthyroid patients did not differ much from the normal range used at the hospital (Table III) The sl ghtly greater range in the euthyroid group could only to a certain extent be explained by an earlier treatment of the thyroid. However the mean value in the untreated group was not significantly different from the mean value in the untreated group for any of the thyroid function tests.

The diagnostic accuracy defined as the percentage of a group whose thyroid state would b correctly diagnosed—true diagnosis—by means of a given thyroid function test, was determin d In determining the validity of a test one is interested not only in the diagnostic accuracy but also in how many cases in the different function groups *are given a false positive diagnosis on* the basis of the results of the laboratory test. Here false positive diagnosis is taken to mean a diagnosis based on the results of a certain test which is not true according to the clinical diagnosis. The percentage of false positi e diagnoses is calculated as the number of patients with false positive diagnosis in per cent of the number of patients in an adjacent diagnostic group or groups. The number and percentage of true diagnoses and false positive diagnoses of thyroid function with the different l boratory tests are given in Table IV No overlapping oc curred b tween the hyperthyroid and hypothyroid groups in any of the tests. Comparisons were made in two groups one group included all patients examined on the basis of PBI and the other all patients examined on the ba is of T_3

It was found that BMR gave a very high diagnostic accuracy for hyperthyreosis, but t the same time a rather high percentage of false positive diagnoses of hyperthyreosis. Reflex time ga e in both groups a high diagnostic accuracy but at the same time a much lower percentage of

Table IV *The frequency of true and false positive diagnoses in the different thyroid function groups with different thyroid function tests*

Basal metabolic rate (BMR), reflex time, protein-bound iodine (PBI) and [131]iodine uptake after 24 h (T_{24}). Percentage of false positive diagnoses calculated as number of patients with false positive diagnosis in per cent of number of patients in adjacent diagnostic group or groups. Normal ra ges – Table III

	True diagnosis		False positive diagnosis		True diagnosis		False positive diagnosis		True diagnosis		False positn diagnosis	
	No.	Hyper thyroids (%)	No.	Euthy-roids (%)	N	Euthy-roids (%)	No.	Hyper- and hypo-thyroids (%)	No.	Hypo-thyroids (%)	No	Euthy-roids ( )
BMR	14	100	12	23	36	68	2	9	7	78	5	9
Reflex time	11	79	66	11	32	60	3	13	9	100	15	28
PBI	10	71	5	9	40	76	7	30	6	67	8	15
BMR	23	96	17	27	41	66	2	6	8	89	4	7
Reflex time	20	83	10	19	37	60	4	12	9	100	14	24
T_{24}	13	54	10	19	41	66	13	39	6	67	12	19
Reflex time + BMR	11	79	2	4	51	96	5	22	7	78	0	0
BMR + PBI	10	71	2	4	50	94	8	35	5	56	1	2
Reflex time + PBI	8	57	1	2	49	93	7	30	8	89	3	8
Reflex time + BMR	19	79	5	9	57	92	6	18	8	89	0	0
BMR + T_{24}	14	58	4	8	57	92	14	42	5	56	1	2
Reflex time + T_{24}	11	46	4	8	54	87	15	46	7	78	4	8

false positive hyperthyroids than BMR. No statistically significant difference was found between reflex time, BMR and PBI concerning the diagnostic accuracy for hyperthyreosis. The diagnostic accuracy of BMR was probably significantly ($p < 0.05$) greater than that of T_{24}.

The number of hypothyroids was fairly small, and the diagnostic accuracy between the tests differed very little. Reflex time gave, however a high percentage of false positive diagnoses of hypothyreosis.

The diagnostic accuracy in the euthyroid group seemed to be somewhat higher for PBI than for BMR and reflex time, and about the same for T_{24} as for BMR and reflex time, but no statistically significant difference was obtained. A high percentage of patients with false positive diagnosis of euthyreosis was obtained with PBI and T_{24}. All false positive diagnoses of euthyreosis made according to reflex time originated from the hyperthyroid group.

The diagnostic accuracy in the euthyroid group increased for PBI and T_{24} if the untreated patients only were taken into consideration, and was greater than the diagnostic accuracy of BMR and reflex time (Table V). The percentage of false positive diagnoses of euthyreosis decreased for PBI and T in the untreated group compared with the whole euthyroid group, and was about the same as for reflex time, but higher than for BMR. A rather considerable increase in diagnostic accuracy in the untreated hyperthyroid group was obtained for T_2 The percentage of true diagnoses in the hyperthyroid group of un treated patients was highest for BMR, and about the same for PBI reflex time and T_{24}

Using a range of ± 1 S.D from the mean of the results in the examined euthyroid group (Table II) the diagnostic accuracy and percentage of false positive diagnoses did not differ much from the results, using the normal range (Tabl III), in any test. A greater diagnostic accuracy was, howeve obtained in the euthyroid group with considerably fewer false positive hypothyroids when 259 msec reflex time was used as the upper normal limit for euthyreosis. The number of true diagnoses of hypothyreosis did not change much.

Statistically significant correlations exist be tween the thyroid function tests used. The highest correlation in the groups of untreated and treated patients was found between BMR and reflex time with a coefficient from -0.58 to -0.64, depending on which group was tested and studied by simpl linear correlation.

The reflex time and the BMR results combined

Table V *The frequency of true and false positive diagnoses of untreated patients in the different thyroid function groups*

	Hyperthyreosis				Euthyreosis				Hypothyreosis			
			False positive diagnosis				False positive diagnosis				False positive diagnosis	
	True diagnosis			Euthyroid	True diagnosis			Hyper and hypothyroids	True diagnosis			Euthyroids
	No	%	No.	( )	No		No	( )	No	%	No	( )
BMR	11	100	8	22	25	69	0	0	3	100	3	8
Reflex time	8	73	5	14	20	56	3	21	3	100	11	31
PBI	8	73	3	8	30	83	3	21	3	100	3	8
BMR	14	100	7	19	15	63	0	0	3	100		8
Reflex time	11	79	4	17	12	50	3	18	3	100	8	33
T[illegible]	11	79	3	13	18	75	4	24	2	67	3	13

gave a better discrimination between the different thyroid function groups, with a higher number of true diagnoses than when the tests were used separately (Table IV). For a diagnosis of hyperthyreosis the patient should have a BMR value equal to or more than +15% combined with a reflex time equal to or shorter than 215 msec. The diagnostic criteria for hypothyreosis were a BMR value of −10% or less, combined [illegible] a reflex time of [illegible] msec or more. The [illegible] which did not fulfil the abovementioned [illegible] gave the diagnosis euthyreosis. With these [criteria] the diagnostic accuracy was about 80% for hyper and hypothyreosis with, at the same time a low percentage (4—9% and 0% respectively) of false positive diagnoses. About 90% of the euthyroids were diagnosed correctly but about 20% of the hyper and hypothyroids were falsely diagnosed as euthyreosis.

Combination of any of two other thyroid function tests and the abovementioned normal values was more accurate diagnostically than each test considered separately but none of the other combinations gave a better result than that of reflex time plus BMR.

DISCUSSION

The results of this investigation show that properly conducted reflex time measurements could be well compared with other thyroid function tests in respect of diagnostic value. Earlier diverging results as to the diagnostic value of reflex time may be explained by inadequate methodology and ill defined material.

Methodological errors

According to Lawson (15) the contraction phase of the reflex is the most valid measure of thyroid function. The Kinemometer distinctly separates the contraction from the relaxation phase while the Photomotograph only gives an indistinct demarcation between the two phases. The variation of measurement of the *S D* interval will also be small, in contrast to that when the relaxation phase is included, as it has a distinct starting and final point.

The paper velocity of the recording instrument influences the accuracy of the measurement. In most published investigations a very low paper velocity—[illegible]5 mm/sec—has been used which gives a percentually higher variation in the results.

The reliability of the test increases with the number of tracings made. With the exception of Robson et al (23) a single tracing has represented the reflex time thus increasing the methodological error.

Some authors (3 [illegible]) have introduced systematic errors by choosing the shortest reflex of a variable number of tracings.

In earlier investigations only reflexes from one side have generally been recorded. This h[as] probably decreased the validity of the test, as in this investigation a statistically high asymmetry was shown between the two sides. It seem[s] prob[able]

that the mean value of the two sides has a higher validity as test of thyroid function than the shortest or longest reflex time. The asymmetry in patients without unilateral neuromuscular disease is probably an expression of biological variation.

If the distortions of the tracings of the kinemometer are not avoided or are not taken into consideration, a falsely long reflex time is obtained (see Methods)

No notice was taken in previous investigations of all these sources of error A low reliability decreases the ability to distinguish between different diagnoses of thyroid function. This may explain why different authors have obtained different results as regards the possibility of using reflex time as a useful test of thyroid function.

The composition of the material

Diverging results might also depend on varying composition of the material, especially as regards previous or present treatment of the thyroid gland. The selection of patients in this investigation may be considered to represent fairly well the average patient material subjected to evaluation of thyroid function at the Departments of Internal Medicine and Radiotherapy (Table I). Practically all *patients with a newly* discovered thyroid disturbance in the Umeå region are treated as in-patients in these Departments. To a large extent, owing to geographical factors among others, the check-ups of non-surgical treatment are also done in the wards.

Comparison between thyroid function tests

It was of interest to compare reflex time with PBI, ^{131}I uptake and BMR as thyroid function tests in this group of patients which was heterogenous as far as thyroid and extra-thyroid factors affecting thyroid function tests were concerned. The group included patients who had had treatment of the thyroid gland—about third of the investigated group—as well as those who had had no such treatment. Treatment of the thyroid may influence the PBI value and the radioiodine uptake *in a diagnostically* misleading way (14 16 17 28).

The BMR value is affected by several diseases despite normal thyroid function. As the mean age was 50 many subjects may be expected to have had dis ses other than c ance of the thyroid function

The effect of drugs and extra-thyroid dis on thyroid function tests illustrates the imp t of developing new diagnostic method tl affected only by altered thyroid fur t not by changes in the biological envir nr normal range for the reflex time o t seems to be unaffected by earlier tr t the thyroid gland—as is also BMR— d factors related to the selection r the investigated hospital group

According to some authors (l l neuromuscular diseases lengthen t t Other authors (5 15) ha e f nd such a change in reflex time in when compared with patients w t eases. In the examined group o were, however rather few with involvement of the calf l ence in reflex time between the euthyroid patients and th negligible in this investigation.

The distribution in the number o case true diagnosis and false positive diagnosis n thyroid function group is affected by what considered the normal range. If there is a wide normal *range, most of the normals* fall within the limits, but there will also be more false positive euthyroids, i.e. the test does not very well separate the pathological from the normal cases. If the normal limits are narrow most of the pathological cases will fall outside, but so will also many normal cases, i.e. there will be many false positive hyperthyroids and hypothyroids.

In the presented material, and with the earlier mentioned normal values, about the same total number of true diagnoses was obtained with BMR, reflex time and PBI in the three thyroid function groups together BMR gave the highest number of true diagnoses in the hyperthyroid group reflex time in the hypothyroid and PBI in the euthyroid group BMR as well as reflex time, in comparison with T_3 gave a higher diagnostic accuracy in the hyperthyroid and hypothyroid groups, *with predominance for* BMR and reflex time respectively In the euthyroid group the three tests showed about the same result as regards true diagnosis. If untreated patients only were taken into consideration, reflex time showed

a somewhat lower number of true diagnoses in the whole group compared with BMR, PBI and T_{24}.

A clinician may however obtain more information from a test than from the relation of the value to a fixed normal range test, because of his knowledge of the patient, of the patient's disease, and of the influence of different sources of errors on the test results. The radioiodine uptake and PBI can also in many cases be improved by additional measurements (16, 17).

Combination of reflex time and BMR for diagnosis of thyroid function

Two tests with the same reliability and validity are not mutually exclusive, but may complement each other. Of the tests discussed here, PBI and ^{131}I uptake are indirect measures of the iodine metabolism in the thyroid, while BMR and reflex time are different expressions of the peripheral effects of the thyroid hormone. The coefficient of correlation is highest between BMR and reflex time even though only 35 % of the total variation in BMR can be predicted by changes in reflex time. As the combined criteria of these two tests proved to give more information about [illegible] function than each test used separately [illegible] do not seem to measure identical peripheral [illegible] of the thyroid hormone.

As the hyperthyroids and hypothyroids are small in number compared with the euthyroids, even in a selected hospital group it is most important that the diagnostic accuracy of the euthyroids is highest. The number of false positive diagnoses should then be least. To distinguish the pathological cases from the euthyroids, the diagnostic accuracy of hyper- and hypothyroids can, however, not be allowed to be much smaller than for the euthyroids. A diagnostic accuracy of 90% in euthyroids, with at the same time true diagnosis of eight out of every ten hypothyroids or hyperthyroids found with the combined criteria for BMR and reflex time, seems to give a satisfactory diagnostic accuracy for all thyroid function groups.

As, for euthyroids, the limits for reflex time and BMR were least affected by treatment of the thyroid gland (Table III) and both are peripheral expressions of hormonal effect, the combination of these two tests is particularly useful for evaluating treated thyroid patients or for following the effects of different thyroid treatments. The measurement of reflex time does not substitute other tests but seems to be a valuable contribution to the arsenal of thyroid function tests.

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ASSOCIATION EUROPÉENNE DE MÉDECINE INTERNE D ENSEMBLE

Le 16 novembre 1968 à Bruxelles, les internistes du Comité de la Section de Médecine interne de l'Union européenne des Médecins spécialistes (UEMS) avait étudié un projet de création d'une organisation de la médecine interne qui aurait pour champ l'Europe entière fut alors constitué un groupe de neuf membres fondateurs.

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s *Pr J Stahl* et *Dr J D gnel*
Président Secrétaire

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A SLOW MOVING PRE-β-LIPOPROTEIN BAND IN SERUM

Report of a Diabetic Serum Subjected to Lipoprotein Electrophoresis

L. E. Wille

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Abstract. On studying blood lipids in diabetics one of the patients showed an abnormal pre-β-band with slow electrophoretic mobility. This was observed with a number of paper electrophoretic techniques and was also found after ultracentrifugation of the serum. This type of slow moving pre-β-band on paper electrophoresis has not previously been reported. The abnormal pre-β-band was present even though the serum triglycerides were also normal.

Variation in the electrophoretic mobility of the pre-β LP fraction on paper has not previously been described. On studying the blood lipids in diabetics (10) one of the patients showed a pre-β-band differing from the normal pre-β's, having a slower electrophoretic mobility. This observation suggested a more thorough investigation of this slow moving pre-β-LP fraction. In the present work this fraction has been studied by different electrophoretic techniques as well as by ultracentrifugation. The electrophoretic mobility has also been considered in relation to lipid composition of the serum.

CASE REPORT

The patient studied is a 20-year-old woman with well regulated diabetes mellitus. She has had diabetes from the age of 15 being treated with diet and insulin (Swine insulin, 8+3 N.U. daily). She has not yet evolved any complications from the diabetes, having normal renal function, no hypertension or atherosclerosis, and no diabetic retinopathy or neuropathy. In addition to the diabetes the patient has a chronic polyarthritis complicating her case. The polyarthritis started at the age of 7 and shows no signs of activity. ESR = 12 mm/h, AST and Waaler test are normal.

METHODS

Lipoprotein electrophoresis was mainly performed on paper according to Lees and Hatch (6). The cell was equilibrated for at least one hour. The strips were run for 22 hours at 0.5 mA per strip and heat-denatured at 95°C for 20 min. The strips were stained for 30 min in Sudan Black and then washed 3 times in 50% ethanol for 2 min. Lipoprotein electrophoresis was also performed on cellulose acetate according to Chin and Blankenhorn (2).

The ultracentrifugation of serum was done in the Ti-50 rotor of a Spinco Model L-65-B at 40,000 rpm (105,000 g) for 17 hours at density 1.006.

Serum cholesterol was determined according to Carr and Drekter (1) and serum triglycerides according to Laurell (5).

RESULTS

In Fig. 1 is presented lipoprotein electropherogram from serum of the case reported. The slow moving pre-β is here denoted type *b*. It has been run side by side with the usual pre-β-LP in serum for comparison (denoted). Pre-β from the patient shows a reduced length of electrophoretic mobility.

The slow mobility of the pre-β-LP was confirmed on hanging strips (Fig. 1), on horizontal strips (Fig. 2) and on lipoprotein electrophoresis performed on cellulose acetate (Fig. 3).

Fig. 4 shows the electrophoretic mobilities of LP found in the super- and infranatants after ultracentrifugation of the patient's serum at density 1.006. It is clearly shown that the supernatant fraction is of pre-β LP nature and not of β-nature which is found in the infranatant.

The concentration of triglycerides and cholesterol in 1.006 supernatant, 1.006 infranatant and 1.006 middle layer are presented in Table I. Analysis of the lipid components in the three fractions isolated by ultracentrifugation showed almost normal values in accordance with those

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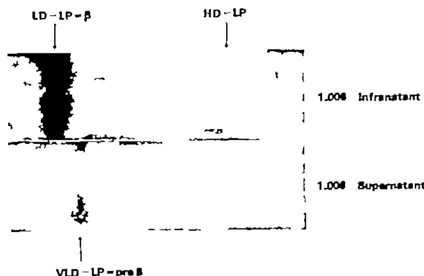


Fig. 4. Electropherograms of the supra- and infranatants after ultracentrifugation of the patient' serum at density 1 006. The lipoprotein electrophoresis performed on paper in Durrum cell with "hanging" strips.

given by de Lalla & Gofman (4) (Table I). The only significant deviation was a lowered cholesterol content in the supernatant, 7.5% against 15% in a normal group.

Fig. 5 presents quantitative estimation of the patient's serum protein fractions after electrophoresis on cellulose acetate. There is a slight increase in the α_1-fraction (11%). This increase might possibly be related to the mild chronic polyarthritis of the patient, indicating a moderately active inflammation process.

DISCUSSION

The present demonstration of slow moving pre-β-LP in serum by a number of different techniques involving paper electrophoresis has not previously been reported. It should, however be mentioned that Noble has described at least three forms of the pre-β-lipoprotein band on agarose electrophoresis (8), one which moves slowly one with an intermediate electrophoretic mobility and a fast moving type. The slow moving pre-β on paper described in this communication might possibly be similar to the slow moving fraction on agarose.

Many factors influence the electrophoretic mobility of the pre-β fraction in serum. The structure of the protein components, the apolipoproteins, is regarded as most important in determining the electrophoretic mobility (3). The composition and the structure of the very low density lipoprotein (VLD-LP) may vary in diseases such as diabetes mellitus and other patho-

Table I. *The distribution of cholesterol and triglycerides in different lipoprotein fractions obtained after ultracentrifugation of serum from the patient*

	Cholesterol		Normal values (Gofman)	TG		Normal values (Gofman)
	(mg%)	( )	(%)	(mg)	( )	(%)
1 006 supernatant – VLD–LP (pre-β)	18	7.5	15	33	64	56
1 006 middle layer	30	1_5		12	23	
1 006 infranatant – LD–LP + HD–LP (β)	193	80	64 (18+46)	7	13	18
Total serum	240			52		

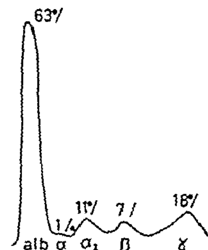


Fig. 5. Serum protein fractions after protein electrophoresis on cellulose acetate.

logical conditions (10). It has been shown that the variation of high density lipoprotein (HD-LP) in the pre-β-fraction influences its mobility in an electrophoretic field (7). Also the plasma free fatty acids (FFA) as well as the saturation of lipids on the apolipoprotein influence this mobility (9).

Usually a hyper-pre-β-lipoproteinemia is related to a hypertriglyceridemia. In the present the TG-values were normal (Table I), but still a pre-β was visible. This phenomenon has been previously demonstrated in other patients with diabetes mellitus (10).

The lowered content of cholesterol in the 1.006 supernatant after ultracentrifugation of the serum could agree with varying composition of the VLD-LP fraction during some diseases such as diabetes mellitus. This may also be related to the decrease in the electrophoretic mobility.

Patients with the usual pre-β-band of the fast moving type often have a significant decrease in the high density lipoprotein class (HD-LP) the quantity of VLD-LP and HD-LP being reciprocal. In the present case the α-band on lipoprotein electrophoresis did not deviate from the normal (Fig. 4) with a concurrent lowered content of HD-LP in the pre-β-band. This possibly implies a decrease in the mobility as postulated for the slow moving pre-β.

In preliminary studies (to be published) we have observed an abnormal distribution of the phospholipids in the serum of the patient. While the total concentration of serum phospholipids was normal, an increase in the cephalin fraction with a concurrent decrease in the lecithin fraction was observed. Similar observations have been made in diabetic sera with a fast moving pre-β-band (type a) upon electrophoresis. The slow moving pre-β-band in serum from the present patient, therefore, cannot be attributed to this abnormal distribution of phospholipids. To conclude a number of factors must be taken into consideration in explaining the electrophoretic mobility of the serum pre-β-band. A possible clinical significance must await further studies of the serum protein and lipid components involved.

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KIDNEY PRESERVATION WITH PULSATILE AND NON-PULSATILE HYPOTHERMIC SERUM PERFUSION

Renal Clearances in Pigs with Autotransplanted 24-hour Preserved Kidneys

H. Løkkegaard, N. Gyrd-Hansen, R. Iversen Hansen, E. Hasselager, B. Nerstrøm and Folke Rasmussen

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Abstract. Preservation of pig kidneys for 24 hours at 8°C has been studied using continuous perfusion with cryoprecipitated, microfiltered serum with pH of 7.67, an oxygen tension of 520 mm Hg and a flow rate of 0.2 ml/g kidney tissue/min. Twenty consecutive experiments were performed. In all cases rapid onset of urine flow was seen after autotransplantation of the kidney and histological examination one hour after recirculation showed only minor tubular damage in most cases. Nine animals died from complications unrelated to the perfusion. Two animals died from uraemia due to technically deficient perfusion (clamping of the renal vein and perfusion with lipaemic serum). With apparently optimal technique three animals died from cortical necrosis whereas six survived and were observed for three months. Measurements of inulin, endogenous creatinine, urea and PAH clearance 10, 31 and 94 days after transplantation in these six animals showed normal values as compared to a control group of autotransplanted, not long-term preserved pig kidneys. There was no difference in the results using pulsatile and non-pulsatile flow.

When immediate function of the implant is wanted, kidney preservation for more than a few hours requires apparatus for continuous perfusion.

Hypothermic storage without continuous perfusion limits the time of safe preservation to about eight hours (4, 5, 24). Attempts to supply the kidney with oxygen by diffusion from the surface, using storage under hyperbaric oxygen, has not resulted in immediate and fully reversible kidney function in experience with 24 and 48 hours preservation (1, 13, 15, 18, 19, 22, 23).

In hypothermic continuous perfusions the chief purpose of the perfusate medium, apart from cooling the organ, is to supply the kidney with oxygen and nutrients, remove carbon dioxide and other waste products and maintain a constant and physiological pH in the tissue.

Different perfusate media have been used. Blood and blood dilutions may cause vascular damage due to deposits of fibrine and thrombocytes in the small vessels during the perfusion, leading to increase in the arterial pressure or decrease in the flow rate, which reduce their value as perfusate media (9). Considering cell-free media, only plasma or serum allows kidney perfusion for several days without changes in flow and pressure (3, 11). In 1963 Humphries et al. (10) described the first experiments with plasma and serum perfusion, but consistently good results were first achieved when Belzer et al. (3) introduced cryoprecipitated, microfiltered plasma as perfusate medium.

Due to varying opinions concerning the oxygen consumption of hypothermic kidneys (14, 21), the oxygen tension in the perfusate has been varied from about 150 to 2000 mm Hg. Until now hyperbaric oxygenation of the perfusate medium has not resulted in a better preserving effect (8, 9).

Considering in particular the oxygen consumption and the pH at 8°C, the purpose of this work is to investigate the effect of hypothermic serum perfusion in 24-hour kidney preservations and to compare the use of pulsatile and non-pulsatile flow.

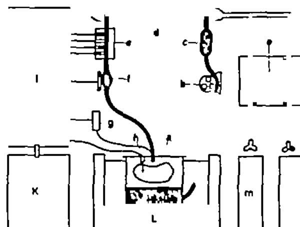


Fig. 1. Schematic representation of the perfusion circuit. *a*, kidney; *b*, roller pump; *c*, filter; *d*, membrane oxygenator; *e*, electrode chamber with pO_2, pCO_2 and pH electrodes; *f*, pulsatile pump; *g*, pressure transducer; *h*, thermistor; *i*, amplifier; *k*, chart mover; *l*, cooling system, *m*, oxygen, *n*, carbon dioxide; *o*, flowmeters.

MATERIAL AND METHODS

Twenty female pigs of the Danish Landrace breed, 4 to 5 months of age, and weighing 47 to 60 kg at the time of surgery were used for the study. The average kidney weight was 136 g (90–168). The animals were fed during the experimental period with a standard fodder mixture (6). They were weighed once a week, and the daily administration of fodder was calculated on the of the body weight. Unlimited quantities of water permitted.

Preservation of the kidney

A schematic representation of the perfusion circuit is shown in Fig. 1.

Two pumps, a pulsatile pump (Harvard Model 1405) and roller pump (Watson-Marlow MHRE) permitted perfusion with pulsatile as well as non-pulsatile flow. The flow rate was adjusted by means of the roller pump.

The respiratory part of the system consisted of.

1. A membrane oxygenator (constructed by Dr S. O. Dawids) which was able to increase the oxygen tension in the perfusate to about 520 mm Hg;

2. An electrode chamber (Radiometer type D566014) with pO_2, pCO_2 and pH electrodes (Radiometer type E5046, E5036, G265 and K567053) connected to Gas-monitors (Radiometer type PHA927*b*) allowing continuous measurements in the perfusate of the values mentioned. The electrode chamber was connected to the cooling system, and the measurements were carried out at the same temperature as that at which the kidneys were preserved.

3. Two flowmeters (Fischer and Porter type 10A1017) which permitted measurement of CO_2 (0–40 ml) and O_2 (0–900 ml) flows with great accuracy. Adjustment of an exact pH value in the perfusate was possible by varying the amount of CO_2 supplied to the membrane oxygenator.

The cooling system (Heto ultra-cryostate type CO) kept the temperature in the kidney in the range of 7–9°C. The temperature was controlled by means of thermistor (Yellow Springs type 524) placed about one cm inside the kidney.

The arterial pressure was measured about 5 cm from the hilum by means of a pressure transducer (Statham type P23AA).

The arterial pressure, pO_2, pCO_2, pH and the temperature were registered continuously on chart mover (Beckman type 504D).

The urine production during the perfusion was not separated from the perfusate medium. The perfusate medium was continuously filtered (Leuko-Pak® Leucocyte Filter) during the perfusion.

The perfusate medium was pooled homologous serum. After coagulation the blood was centrifuged for 30 min at 2000 rpm (1326 g). The serum was siphoned off and then stored at −20°C. Immediately before use the serum was thawed and filtered as described by Belzer et al. (3). Before filtration 20 mg gentamycinum NFN (Garamycin®) and 25 mg papaverini sulfas NFN were added to each 500 ml serum, which was the volume used for the perfusion.

Five minutes before removal of the kidney the animal was heparinized (5000 I.U. heparinum NFN per 10 kg body weight). As soon as possible after removal (2.5 min) the kidney was perfused with TIS-U-SOL as described earlier (15). The kidney was weighed and then placed in the perfusion circuit. On the basis of the weight of the kidney the roller pump was adjusted to give flow rate corresponding to 0.2 ml/g/min. The pH was adjusted to 7.67 and kept in the range of 7.60–7.70 during the perfusion. Twenty-four hours later the kidney was removed from the perfusion circuit, weighed and reimplanted in the animal. One hour after recirculation biopsy was taken from the kidney. The concentrations of sodium, potassium, bicarbonate, protein and the activity of lactic acid dehydrogenase in the perfusate medium were determined before and after the perfusion.

Surgical technique

Renal autotransplantation with uretero-ureteral anastomosis was performed followed by contralateral nephrectomy immediately after transplantation. Details of the surgical technique have been published previously (7).

Post-operative Studies

Blood analyses

During the first week after the operation blood samples were taken every other day and subsequently once week for the following three months. The pH and haematocrit values were determined, together with the concentrations in plasma of creatinine urea, sodium and potassium.

Kidney function

The clearances of inulin, endogenous creatinine, urea, para-aminohippuric acid (PAH), and the excretion

Table I. *Experimental data from 20 hypothermic serum perfusions of pig kidneys*

From pig no. 60 to 75 pulsatile flow and from pig no. 76 to 84 non-pulsatile flow has been used

Pig no.	Flow (ml/min)	Perfusion pressure (mm Hg) Start	Perfusion pressure (mm Hg) End	Weight gain (%)	Potassium (mEq/l) Start	Potassium (mEq/l) End	Sodium (mEq/l) Start	Sodium (mEq/l) End	Bicarbonate (mEq/l) Start	Bicarbonate (mEq/l) End	LDH (normal 44±6) Start	LDH (normal 44±6) End
					Biochemical changes in the perfusate during the perfusion							
60	30	38/22	36/18	14	5.3	8.2	138	135	17.5	19.2	74	77
62	28	28/14	28/12	8	5.9	7.1	143	135	24.9	23.1	46	50
63	22	38/22	38/24	24	5.7	7.2	142	139	20.8	21.8	64	62
64	26	44/30	36/22	15	6.6	8.8	145	141	23.0	31.3	37	47
65	27	20/12	18/10	13	6.4	7.7	142	136	25.8	22.0	54	54
66	22	38/26	32/22	25	5.2	8.5	142	138	23.3	20.3	41	54
67	31	40/24	28/14	—	—	—	—	—	—	—	—	—
68	23	40/28	20/14	20	8.3	11.7	144	140	15.9	15.9	58	76
69	21	40/24	28/14	30	6.0	8.8	143	139	16.5	16.3	50	51
70	24	26/16	32/22	25	3.6	6.1	141	137	26.4	26.6	71	71
72	18	30/16	26/16	18	4.6	9.8	137	129	23.6	22.0	52	65
74	27	46/28	28/10	24	6.1	11.2	134	130	17.3	18.8	55	62
75	34	34/18	18/8	13	4.9	9.3	134	134	18.6	17.9	45	50
76	33	30	16	10	5.6	5.4	145	144	18.6	16.2	36	67
78	25	35	10	11	5.2	8.2	136	134	23.8	25.2	—	69
79	27	26	18	31	5.2	9.4	145	144	14.8	12.6	86	100
81	30	24	14	12	5.1	9.4	141	135	20.5	24.9	56	63
82	32	20	8	18	5.3	9.0	135	130	13.3	14.7	74	63
83	28	30	18	25	4.4	7.0	138	133	20.5	25.3	26	33
84	32	50	30	24	3.6	8.9	137	134	20.1	22.5	67	80

percentages of water sodium, potassium and chloride were determined. The clearance measurements were performed on unanaesthetized animals 10, 31 and 94 days after transplantation. Each experiment comprised at least three periods of 20 min, and the last experiment was concluded with three periods of 20 min to determine the Tm for PAH. Three days after the last clearance experiments the extraction percentage for PAH was determined, after which the animals were killed. Details of the doses of test substances, the technique and the calculation methods have been published previously (7).

Analytical methods

The bicarbonate concentration was determined by the method of Jørgensen and Astrup (12). The other methods have been described previously (7 15).

Postmortem examinations

The animals which survived during the whole observation period were killed and bled, and postmortem examination as performed. The animals which died from complications ere examined as soon as possible after death. The kidneys were weighed.

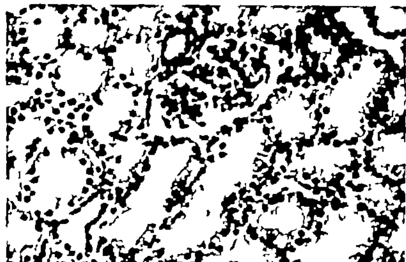
Histological examination

At necropsy kidney tissue was removed and fixed in neutral buffered formalin. The biopsies taken one hour after recirculation were fixed in Zenker' fluid and in neutral buffered formalin. Paraffin wax sections were stained ith iron haematoxylin-van Gieson, and the periodic acid Schiff reaction was carried out according to McManus and Mowry (20).

RESULTS

The perfusion

Table I summarizes the most important data. Using a flow rate of 0.2 ml/g/min the arterial pressure in no case exceeds 50 mm Hg. With the exception of one perfusion (pig no. 70) the pressure decreased during the perfusion. The weight gain during the perfusion was on verage 18% without significant difference between pulsatile (19%) and non-pulsatile flow (17%) The aver age increase of the potassium concentration in the perfusate was 3.1 mEq/l corresponding to a loss of about 15% of the intracellular potassium of the organ. The potassium loss was identical in experiments with pulsatile and non-pulsatile flow The increase in the potassium concentration of the perfusate corresponded to a decrease in the sodium concentration of 4.0 mEq/l. Before and after the perfusion the concentration of protein in the serum was unchanged and on average 5.7 g/100 ml. The relative distribution between the different protein fractions determined


A


B


C



Fig. 2 (A) Histological section of kidney biopsy from normal pig kidney. (B) Histological section of kidney biopsy from pig kidney after perfusion for 4 hours. More indistinct and granular cytoplasm in the tubular cells. Dilatation of glomerular spaces. (C) Histological section of kidney biopsy from pig kidney one hour after recirculation. Normal tubule (*a*). Pyknotic nuclei and desquamation (→). (D) Histological section of pig kidney at autopsy three months after transplantation. Hypertrophy of glomerulus and tubules. Slight dilatation of glomerular space. Zenker fixed. Iron haematoxylin—van Gieson, 250.

by paper electrophoresis was likewise unchanged (alb. 47% glob., α_1 13% α_2 16% β 15% γ 19%). The LDH activity increased on average 17%.

Initial behaviour

After removal from the perfusion circuit an average of 37 min (31–77) passed before recirculation was established. Immediately after the recirculation all the kidneys turned pink, with the exception of the kidney from pig no. 67 and the urine production started within a few minutes. In three cases (pigs nos. 66, 72 and 82) the pink colour remained unchanged during the surgery but in all other cases shifting blue areas, starting 3–15 min after recirculation, were seen on the surface of the kidneys. With the exception of pigs nos. 67, 69, 72 and 82 (described later) histological examination of 16 kidneys one hour after recirculation revealed a picture as exemplified in Fig. 2 C. No pathological changes were found in the glomeruli. In the proximal tubules varying degrees of cellular desquamation, pyknosis of the nuclei of the cells and hyalin degeneration were found. In most cases pyknosis of the nuclei did not exceed about 5% of the nuclei of the proximal tubules. In Fig. 2 the histological examination one hour after recirculation is compared with histological examinations before removal of the kidney (Fig. 2 A) and after 24 hours serum perfusion (Fig. 2 B). After 4 hours serum perfusion the glomerular spaces were slightly dilated and granular cytoplasm was found in the tubular cells.

After the transplantation fourteen pigs died of various complications and six pigs survived the observation period (3 months).

Complications

Pigs nos. 62, 64, 65, 68 and 74 died due to surgical failures (thrombosis of the renal artery and stenosis of the ureter).

Pig no. 67. Just before the recirculation the clamp was removed from the vein by accident, and the kidney was filled with blood from the venous side. When the arterial clamp was removed only half of the kidney turned pink, while the rest remained blue. The histological examination one hour after recirculation showed that the capillary loops in about half of the glomeruli were occluded by fibrinous thrombi. The pig died one day after the transplantation. Postmortem examination revealed necrosis of the renal cortex without thrombus formation in the artery and vein.

Pig no. 69. After 23 hours in the perfusion circuit the kidney turned oedematous, and the arterial pressure rose from 20 to 60 mm Hg due to the fact that the vein was suddenly occluded by external pressure. Five minutes after the arterial pressure began to rise the failure was corrected. The pressure fell to 20 mm Hg and the oedema decreased, but at the end of the perfusion the weight increase was 31%. The histological examination one hour after recirculation showed that the vessels in the glomerular tufts were dilated. The capillaries in the glomeruli as well as in the interstitium showed a marked increase of the granulocytes. Pyknosis of the nuclei was seen in about 20% of the cells of the proximal tubules. The pig died one day after transplantation. The postmortem examination revealed necrosis of the renal cortex without thrombus formation in the artery and vein.

Pig no. 70 died two months after the transplantation due to intestinal obstruction because of fibrous

Table II *Average renal clearances in six pigs 10 to 94 days after transplantation*

p = pulsatile flow (pigs nos. 60, 63, 66), n-p = non-pulsatile flow (pigs nos. 76, 78, 83). The bracketed figures indicate minimal and maximal values of single experiments

Days after transplantation	Perfusion	Body weight (kg)	Haematocrit (%)	Diuresis (ml/min)	Clearance: Inulin (ml/min/10 kg b wt.)	Clearance: Endogenous creatinine (ml/min/10 kg b.wt.)	Clearance: Urea (ml/min/10 kg b.wt.)	Clearance: PAH (ml/min/10 kg b.wt.)
10	p	52 (47–60)	33 (28–36)	2.1 (0.8–4.1)	11 (10–12)	14 (12 17)	8 (7–9)	49 (44–54)
	n-p	52 (47 58)	32 (31 32)	2.2 (1 7 2.9)	11 (8–13)	14 (9–16)	7 (5–8)	50 (37–61)
31	p	70 (61–83)	39 (34–43)	1.6 (1.0–2.7)	16 (15–18)	17 (16–19)	10	58 (53–66)
	n-p	70 (65–76)	40 (37–44)	1 7 (1.6–1.8)	14 (13–16)	14 (15–16)	9 (8–10)	55 (53–58)
94	p	116 (110–125)	45 (43–46)	2 4 (1 6–3.2)	16 (15–18)	16 (15–19)	10 (8–11)	55 (41–71)
	n-p	119 (105–139)	39 (36–44)	3.6 (1.9–5.8)	18 (15–22)	19 (16–25)	10 (7–12)	66 (55–84)

adhesions. Plasma-creatinine was 11.9 μg/ml, and inulin, endogenous creatinine, urea and PAH clearances wer 20, 21, 13 and 5? ml/mm/10 kg body weight, respectively Macroscopic and microscopic examinations of the kidney showed no pathological changes.

Pig no. 72: The time for suturing the vessels was 77 min. The biopsy one hour after recirculation showed focal increase in the granulocytes of the capillaries. Pyknosis of the nuclei was found in about 10% of the proximal tubular cells. The pig died three days later without any urine formation. The postmortem examination revealed necrosis of the renal cortex without thrombus formation in the artery and vein.

Pig no. 75 died twelve days after transplantation due to intestinal obstruction because of entrapment of bowel in hernia. The inulin, endogenous creatinine,

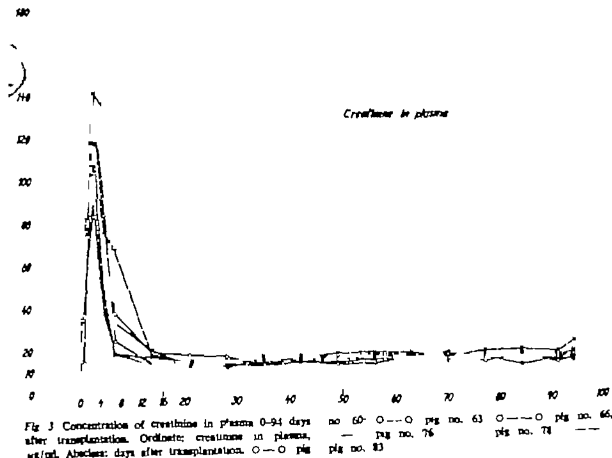


Fig 3 Concentration of creatinine in plasma 0–94 days after transplantation. Ordinate: creatinine in plasma, μg/ml. Abscissa: days after transplantation. O — O pig no 60- O -- O pig no. 63 O — — O pig no. 66, — pig no. 76 pig no. 78 — — pig no. 83

		Clearance ratios		
Effective renal plasma flow (ml/min/10 kg b.wt.)	Effective renal blood flow (ml/min/10 kg b.wt.)	Cr/I	Urea/I	Filtration fraction In/PAH
53 (48–59)	79 (72–88)	1.3	0.7	0.22
54 (40–66)	79 (59–92)	1 3	0.6	0.22
63 (58–72)	103 (95–118)	1 1	0.6	0 28
60 (58–63)	100 (97 105)	1 1	0 6	0.25
60 (45–77)	109 (82–140)	1.0	0.6	0.29
72 (60–91)	118 (98–149)	1 1	0.6	0.27

urea and PAH clearances on the tenth day after transplantation were 10, 13, 7 and 53 ml/min/10 kg, which were the same as the average values for the surviving animals on the tenth day after transplantation (Table II). Macroscopic and microscopic examination showed no pathological changes.

Pig no. 79: Although the serum was handled as described it was cloudy probably due to high fat content. The pig died four days after the transplantation without any urine formation. The postmortem examination revealed necrosis of the renal cortex without thrombus formation in the artery and vein.

Pig no. 81 died nine days after transplantation. There was abundant urine formation from the beginning, but due to pronounced loss of sodium the concentration in serum fell to 108 on the day of death. Cadaverous

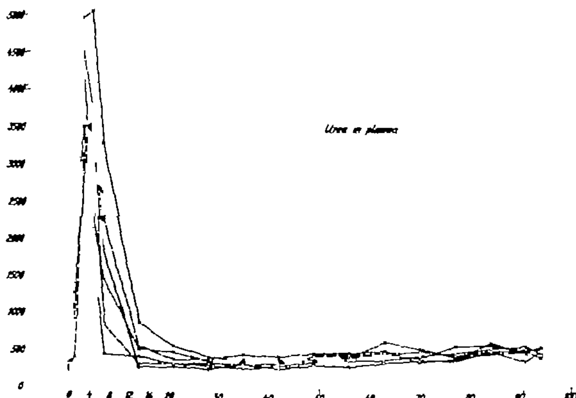


Fig. 4. Concentration of urea in plasma 0–94 days after transplantation. Ordinate: urea in plasma, µg/ml. Abscissa: days after transplantation. O — O pig no. 60· O O pig no. 63 O —— O pig no. 66, — pig no 76, pig no 78 —— pig no. 83

Table III. *Renal clearances and total renal blood flow in six pigs 94 days after transplantation*
p = pulsatile. n-p = non-pulsatile

		Clearance							
		Inulin		Endogenous creatinine		Urea		PAH	
Pig no.	Perfusion	(ml/min/10 kg b.wt.)	(ml/min/100 g kidney)	(ml/min/10 kg b.wt.)	(ml/min/100 g kidney)	(ml/min/10 kg b.wt.)	(ml/min/100 g kidney)	(ml/min/10 kg b.wt.)	(ml/min/100 g kidney)
60	p	15	41	15	50	10	34	54	180
63	p	18	38	19	40	11	24	71	149
66	p	16	35	15	34	8	18	41	91
76	n-p	15	45	17	48	10	28	55	158
78	n-p	18	47	16	41	7	18	59	153
83	n-p	22	39	25	45	12	21	84	149

changes in the kidney prevented a more careful post mortem histological examination.

Pig no. 82: Histological examination one hour after transplantation showed a focal increase of the leucocytes in the capillaries and pyknosis of the nuclei in about 15% of the proximal tubular cells. The pig died one day after transplantation. The postmortem examination revealed necrosis of the renal cortex without thrombus formation in the artery and vein.

Pig no. 84 died one day after transplantation. Post mortem examinations revealed necrosis of the renal cortex without thrombus formation in the artery and vein.

Renal Function in the Six Pigs Surviving Throughout the Experimental Period

Concentrations of creatinine, urea, sodium and potassium in plasma

Figs. 3 and 4 show the concentrations of creatinine and urea in plasma (μg/ml), respectively.

The concentration of sodium and potassium was constant throughout the period: 140 ± 5 (S.D.) and 5.0 ± 0.8 (S.D.), respectively. The pH remained constant, averaging 7.34 ± 0.04 (S.D.).

Clearances of inulin, endogenous creatinine, urea and PAH

Table II gives the average values for the clearances of inulin, endogenous creatinine, urea and PAH, for the effective renal plasma flow ($RPF_{effective}$) and effective renal blood flow ($RBF_{effective}$). The clearance ratios are also stated. It will be seen that no difference in renal function between kidneys perfused with pulsatile and non-pulsatile flow was found. Since the animals were killed immediately after the last clearance experiment, it was possible to calculate the clearance both per 10 kg body weight and per 100 g of kidney tissue. The results are given in Table III, which also shows the extraction percentage for PAH used for calculating the total renal blood flow (RBF_{total}).

Maximal tubular excretion of PAH

In the experiment 94 days after transplantation the Tm of PAH was determined, and the results for each individual pig are shown in Table IV, which also shows the concentrations of PAH in plasma at which the Tm was determined. The Tm values were calculated both per 10 kg body weight and per 100 g kidney tissue and are on average 15 mg/min/10 kg body weight and 38 mg/min/100 g kidney tissue.

Excretion of water, sodium, potassium and chloride

The average excretion percentage for water, sodium, potassium and chloride are shown in Table V. The average values for the whole period were ..1 ± 1.0 (S.D.), 0..3 ± 0.10 (S.D.), 37 ± 10 (S.D.), and 1.13 ± 0.5 (S.D.), respectively.

Postmortem Examination on the Six Pigs Surviving Throughout the Experimental Period

Macroscopical findings

The kidneys from pigs nos. 63, 66, 76 and 78 were greyish-brown and had a normal consistency. Vascular and ureteral anastomoses were without complications. The kidney from pigs nos. 60 and 83 had a greyish-brown colour but were firm in consistency and difficult to decapsulate. The kidney from pig no. 83 was

Extraction percentage	Total renal blood flow (ml/min/10 kg b.wt.)	(ml/min/100 g kidney)
87[a]	119	400
87[a]	161	336
91	91	202
87	111	316
91	126	323
80	178	312

Non-estimated 87 is the average extraction percentage in normal pigs.

hydronephrotic with flattened papillae due to a twisted course of the ureter The ureter from pig no. 60 and the vascular anastomosis in pigs nos. 60 and 83 were without complication.

Microscopical findings

The kidney from pig no 60 showed a pronounced focal interstitial fibrosis in the outer part of the renal cortex, but only slight interstitial fibrosis in the rest of the kidney The kidney from pig no. 83 had focal fibrosis due to pyelonephritis and dilatation of the glomerular spaces. The kidneys from the other pigs showed no pathological changes (Fig. 2 D).

DISCUSSION

Perfusion of kidneys under hypothermic conditions raises series of questions, e.g. the supply of oxygen, the acid base status during hypothermia, addition of vasodilators to the medium and the use of pulsatile and non-pulsatile flow

The oxygen supply has to be sufficient in order to prevent anoxic damage to the kidney Levy (14) measured the oxygen consumption of canine kidneys perfused with whole blood at an arterial pressure of 80–170 mm Hg at various temperatures. He found an oxygen uptake of about 20×10^{-4} ml/g/min at 8 C Using an average pO_2 of 520 mm Hg in perfusions with cryoprecipitated, microfiltered serum with an average flow of 0.. ml/g/min and an arterial pressure from .5 to 40 mm Hg, Lokkegaard (16) has found an average oxygen uptake of the same order of magnitude In all cases the effluent had a high pO_2 (Table VI) Compared to Levy's experiences our results indicate that flow rate of 0.2 ml/g/min is probably sufficient at a pO_2 of about 500 mm Hg in the perfusate

The acid base status in hypothermia has been investigated by Siggaard-Andersen and Egsbæk (26) in experiments with hibernating bats. At 8 C they found the pH to be 7 67 (measured at 8 C) a value which was kept within narrow limits (7 60–7 70) in the present experiments.

Heparinization of the animals before removal of the kidneys and addition of papaverine to the perfusate medium was used because this combination gives a low initial perfusion pressure in experiments with hypothermic rat kidney perfusion (17)

We have not been able to demonstrate any difference in the results using pulsatile and non-pulsatile flow Belzer et al. (3) using a flow rate of 1 ml/g/min, state that better results are obtained with pulsatile flow

Table IV *Clearance and maximal tubular excretion (Tm) of para-aminohippuric acid 94 days after transplantation*

p pulsatile flow n-p non-pulsatile flow

Pig no.	Perfusion	Body weight (kg)	Kidney weight (g)	Relative kidney weight ( )	Plasma concentration of PAH (µg ml)	PAH clearance (ml/min/10 kg b)	Inulin clearance (ml/min/10 kg b. t)	Tm (mg/min/10 kg b.wt.)	Tm (mg/min/100 g kidney)
60	p	125	376	0 30	1700	22	13	16	53
63	p	110	527	0 48	1100	31	15	18	38
66	p	114	514	0 45	1160	24	12	14	31
76	n-p	139	481	0 35	1150	25	13	14	40
78	n-p	113	436	0 39	1940	21	12	18	47
83	n-p	105	594	0 57	1230	19	10	12	21

Table V *Average renal excretion of water and electrolytes in six pigs 10–94 days after transplantation*
p = pulsatile. n-p = non-pulsatile. The bracketed figures indicate minimal and maximal values of single experiments

Days after transplan-tation	Perfusion	Body weight (kg)	Diuresis (ml/min)	Inulin clearance (ml/min/10 kg b.w.t.)	Excretion (%) Water	Sodium	Potassium	Chloride
10	p	52	2.1	11 (10–12)	3.7	0.37	40	1.6
	n-p	52	2.2	11 (8–13)	3.8	0.23	42	1.1
31	p	70	1.6	16 (15–18)	1.4	0.26	28	1.0
	n-p	70	1.7	14 (13–16)	1.7	0.07	31	0.66
94	p	116	2.4	16 (15–18)	1.3	0.16	27	0.53
	n-p	119	3.6	18 (15–22)	1.7	0.24	26	0.69

Twenty pigs were used in the present experiments.

Eight pigs died from serious surgical complications and one from severe hyponatriaemia due to insufficient replacement of urinary sodium loss.

Five pigs developed total renal cortical necrosis. In one a temporary compression of the vein during the perfusion may be responsible for the complication. In another case the perfusion was carried out with cloudy probably lipaemic serum. Scott et al (25) have described cortical necrosis using fatty opaque plasma. These authors recommend the following procedure: 1) cryoprecipitation 2) centrifugation of opaque samples, removal of the fatty layer on the surface, millipore filtration. In three of our pigs with cortical necrosis no explanation for the development of this complication could be found.

In the remaining six long-term surviving pigs the concentration of creatinine and urea in plasma as well as different renal clearance studies were carried out and compared to results on a control group of autotransplanted, not long-term preserved kidneys, which have been published previously (7).

The concentration of creatinine and urea in plasma increased during the first days after transplantation (Figs. 3 and 4), fell after the 5th day and were stabilized after –3 weeks, which is in agreement with findings of other workers (2, 3). During the period from 20 to 94 days after transplantation the average concentration of creatinine in plasma was 16 $\mu g/ml \pm 1$ (S.D.), while it was $14 \mu g/ml \pm 0.5$ (S.D.) in the control group ($0.0_ < p < 0.025$).

The inulin, endogenous creatinine, urea and PAH clearances on the 10th, 31st and 94th day after transplantation showed no significant difference (on the 0.01 level) compared with the control group. On the 94th day the average values were 17, 18, 10 and 60 ml/min/10 kg body weight, respectively, while the values in the control group were 18, 18, 10 and 69 ml/min/10 kg body weight.

The excretion percentages of sodium, potassium and chloride were almost identical to values obtained in the control group. The maximal tubular excretion of PAH was on average 15 mg/min/10 kg body weight $\pm 2._$ (S.D.), while Tm in the control group was 25 mg/min/10 kg body weight ± 4.4 (S.D.), ($0.01 < p < 0.005$). The extraction percentage (determined on four pigs) was 87 ± 5 (S.D.) which is identical with the values in normal pigs (6).

The average filtration fraction was 0.27 ± 0.03 (S.D.), while it was 0.29 ± 0.05 (S.D.) in the control group ($0.10 < p < 0.20$).

The average ratio between endogenous creat

Table VI. *Investigations of the oxygen consumption in serum-perfused pig kidneys at 8°C, performed by means of measurements of the oxygen tensions in serum on the arterial and venous side of the kidney*

Perfusion no.	pO_2 (mm Hg) Artery	Vein	Flow (ml/g/min)	Oxygen consump (ml/g/min)
20	540	270	0.19	$19.0 \cdot 10^{-4}$
21	530	240	0.20	$27.1 \cdot 10^{-4}$
27	500	350	0.16	$11.2 \cdot 10^{-4}$
28	500	230	0.20	$25.2 \cdot 10^{-4}$
29	540	350	0.30	$26.1 \cdot 10^{-4}$

inine and inulin clearance was 1.05 ± 0.11 (S.D.), while it was 1.09 ± 0.14 (S.D.) in the control group $(0.025 < p < 0.05)$.

In an earlier work (15) we have shown that kidney preservation for 24 hours at 15 ATA oxygen and 5 C without continuous perfusion causes a transient marked reduction in renal function, and three months after the transplantation the function is still reduced to 60–80%. Compared with these results the method described in this paper appears to be superior although the unexpected cases of cortical necrosis require further clarification before the method can be applied in clinical practice.

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MULTIPLE SERUM ENZYME ANALYSES IN CHRONIC ALCOHOLICS

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Abstract. Multiple serum enzyme analyses in 100 chronic alcoholics with a history of heavy drinking revealed that most serum enzyme activities were increased in some of these patients. Increased serum creatine kinase activity characteristic of muscle injury was found in 43 patients. Since cardiac damage could be excluded by other enzyme tests, skeletal muscle damage seems to be common in alcoholic patients. Elevated serum ornithine carbamoyl transferase (OCT), which is specific for liver damage, was found in 56 patients. Serum GOT commonly used for detection of liver damage, seems in alcoholics to be partly released from skeletal muscles and should then be replaced by the more specific OCT for which there is simple method. In alcoholics the increased serum activity of the intramitochondrial enzyme, glutamate dehydrogenase, suggests rather severe liver damage in nearly half of the patients. Serum guanine deaminase (guanase) was pathological less often than the other "liver enzymes". Heavy increased values were encountered often in serum γ-glutamyl transpeptidase but leucine aminopeptidase and alkaline phosphatase were less often elevated.

There is an established association between excess alcohol consumption and the occurrence of liver damage. The histological picture of these hepatic lesions are variable (19 23 51 81 88, 90). Fat accumulation and mitochondrial changes in liver cells can be provoked by a moderately short drinking period (64 80), and a release of enzymes from the liver has been noticed after a single dose of ethanol (15 34). Further progress of hepatic damage may lead to acute alcoholic hepatitis with intrahepatic cholestasis (10, 11 73) and special changes in serum enzyme activities.

In alcoholic patients increased serum activities of the enzymes present in liver parenchyma have been generally accepted as indicative of liver damage (2, 4, 5 14 35 39 54, 62, 79 84). In clinical practice evidence of liver injury is usually derived from aspartate aminotransferase (GOT) or alanine aminotransferase (GPT) determinations. The toxic effect of alcohol may however manifest itself in many other organs besides the liver. There is a definite association between alcoholism and pancreatitis (46, 85) and alcoholic patients appear to have an increased susceptibility to cardiomyopathy (27). Skeletal muscles deserve special consideration, as several authors have described pathological changes in the musculature of alcoholics (24, 26 40 41 42, 52, 71 72, 87) and the activity of aminotransferases (especially GOT) is high in the muscles.

The present study was made in order to estimate the value of various serum enzyme determinations in the detection of liver damage. By means of a series of multiple enzyme analyses the aim was to determine how often and which organs are effected in alcoholics.

MATERIAL

One hundred male alcoholic patients with a history of continuous or periodic heavy alcoholic abuse of several years' duration were studied. All patients had been admitted to a special alcoholics department of Hesperia Hospital, Helsinki, for psychiatric or social reasons during or immediately after heavy bout of drinking. Twenty-two of the patients had delirium tremens. Details of their history clinical and ECG findings are described in another paper (49). The normal values of serum enzyme activities were calculated from control group of thirty healthy laboratory workers and medical students.

METHODS

Serum enzyme activities were analysed from unhemolysed sera separated immediately from blood drawn within 48 hours after admission. The following enzyme activities were measured on the day of collection to avoid possible inactivation by storage:

Table I *Mean values and S.D.s of serum enzyme activities in IU/l of serum (except for amylase) of the 100 alcoholic patients and of the 30 healthy subjects*

Serum enzyme	Subjects	
	100 alcoholics	30 healthy persons
GOT	32.0 ± 27.5	9.9 ± 3.1
GPT	19.. ± 15.3	8.5 ± 3.1
OCT	0.31 ± 0.56	0.17 ± 0.09
Guan	3.11 ± 5.88	1.44 ± 1.79
γ-GT	7.77 ± 12.20	1.48 ± 0.74
GLDH	4.05 ± 5.66	0.93 ± 0.60
CPK	1.28 ± 1.86	0.29 ± 0.27
Amyl	94.6 ± 64.3	80.2 ± 4.1
LAP	17.4 ± 7.3	13.9 ± 3.3
LDH	216 ± 72	143 ± 23
U-LDH	142 ± 48	103 ± 16
APh	38.9 ± 1..4	27.7 ± 7.8

aspartate aminotransferase – *GOT* (50) alanine aminotransferase – GPT (91), glutamate dehydrogenase – GLDH (86) creatine kinase CPK (94), lactate dehydrogenase LDH (59), urea-stable LDH U-LDH (65), the electrophoretic separation of LDH isoenzymes (44) alkaline phosphatase APh (1), and leucine aminopeptidase – LAP (13)

The following enzyme activities, known not to be affected by storage were measured within 1 week from serum samples stored 20°C

guanine deaminase Guan (47), ornithine carbamoyl transferase – OCT (55 56), γ-glutamyl transpeptidase γ-GT (93), and amylase – Amyl (89)

Furthermore the following analyses were made.

serum bilirubin, potassium, iron, magnesium, bromide, cholesterol, triglycerides, free fatty acids, hemoglobin, hematocrit and prothrombin proconvertin activity (Owren).

Enzyme activities are expressed as IU l of serum for all enzymes studied, except amylase, for which unit of the original method were used. The upper 95% confidence limit of the control group served as the upper limit of normal enzyme activity. When values of a given enzyme showed skewed distribution, this was straightened by logarithmic transformation.

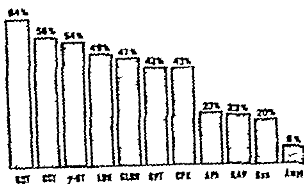


Fig. 1. Pathological serum enzyme activities in 100 chronic alcoholics.

RESULTS

Table I shows the mean values and S.D. of various serum enzyme activities of the 100 alcoholic patients and of the 30 healthy subjects.

Fig. 1 shows how often the serum enzyme activities were pathological in alcoholics. Figs. 2 and 3 present the individual serum enzyme activities generally considered to reflect liver damage and Fig. 4 the other enzyme activities studied.

Figs. 5–7 show the correlation between various "liver enzyme" activities in serum. To make the comparison easier the activities are expressed in relative values. Unity is the upper normal limit, and the further values are its multiplications. As seen from the figures there is a correlation between the values of GOT and OCT (Fig. 5), whereas OCT was augmented more sensitively than guanine deaminase (Fig. 6), and GLDH more than GPT (Fig. 7). No correlation was *observed between the serum enzyme activities* and the kind of alcohol the patients had consumed, nor did the duration of the last drinking bout *seem to affect the results.*

In fourteen patients the liver extended more than 4 cm below the costal margin, and serum OCT was pathological *in all of these patients,* serum GOT was normal in four and γ-GT in two. *In those 47* patients whose liver was tender on palpation, both OCT and GOT showed pathological values in 31 cases. Serum CPK was abnormal in all seven patients who had bruises, and in addition in *36* further patients. *Delirium* tremens was encountered in 22 patients but serum enzyme activities were not more often pathological in these patients than in those without signs of delirium tremens. Bromide was found in the serum of nine patients, indicating that they had ingested bromide containing sleeping pills. These patients did not differ from the others in respect of serum enzyme activities.

In the three patients who had *slightly elevated* serum bilirubin values (3.9 2.6 and 1.8 mg/100 ml) serum γ-GT showed greatly increased values (45.6, 68.7 and 56.3 *IU* respectively), and the two patients who had pitting edema of the ankles had abnormal serum OCT and GOT values.

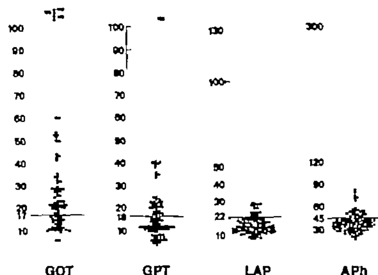


Fig. 2. Serum aspartate aminotransferase (GOT), alanine aminotransferase (GPT), leucine aminopeptidase (LAP) and alkaline phosphatase (APh) activities of 100 alcoholics. The upper normal limit is marked by horizontal line. The values for upper normal limit with 99% confidence are indicated at the lines. Activity expressed as IU/l of serum.

DISCUSSION

The finding that serum GOT was abnormal in alcoholics more than the other "liver enzymes" indicates either that serum GOT more sensitively reflects liver damage or that release of GOT occurs also from other tissues. GOT may be released from skeletal and cardiac muscles, where the activity of this enzyme is about the same as in the liver and where lesions have been reported to occur in alcoholic patients (24 26, 40 41 42, 52, 71 72, 87) That GOT is indeed partly released from the musculature is supported by the fact that in the 19 patients who had pathological serum GOT but normal OCT (OCT is specific for the liver and is not present in the muscles) serum CPK activity was raised in 16 cases. CPK is present abundantly in human skeletal and cardiac muscles (18) and sensitively reflects damage there (21 22, 28, 48, 57 96) That the origin of serum GOT is in part skeletal but not cardiac muscle is supported by the finding that the activity of the slowest moving LHD isoenzymes was increased and that urea-stable LDH activity showed a lowered activity

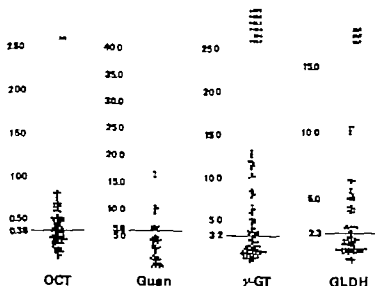


Fig. 3. Serum ornithine carbamoyl transferase (OCT), guanine deaminase (Guan), γ-glutamyl transpeptidase (γ-GT), and glutamate dehydrogenase (GLDH) activities of 100 alcoholics. Other data as in Fig. 2.

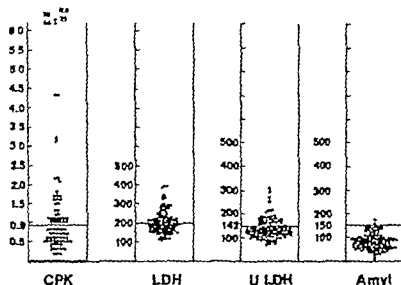


Fig. 4. Serum creatine kinase (CPK), lactate dehydrogenase (LDH), urea-stable LDH (U-LDH) and amylase (Amyl) activities of 100 alcoholics. Serum amylase activity is expressed as mentioned in the text, other activities in IU. Other data as in Fig. 2.

Ornithine carbamoyl transferase (OCT), due to its organ distribution (75) and according to clinical experience (16, 76, 77, 78) seems to be by far the most specific enzyme for the detection of liver damage. Outside the liver this enzyme is present in noteworthy concentrations only in the small intestine, where its activity is 14% of that found in the liver (75). Human cardiac and skeletal muscles and other organs as well as blood cells contain OCT in an activity which is only little more than that of normal serum. Thus OCT seems to offer an optimal test for detection of liver damage. Despite these facts the measurements of OCT has not come into common clinical use, because the determination has been cumbersome or required isotope apparatus. Recently a new simple technique has been developed in our laboratory (55, 56) and this test was used in the present study. That OCT seems to be a more sensitive indicator of liver damage than GOT is supported by the observation that serum OCT was pathological in

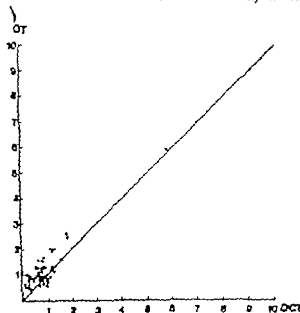


Fig. 5. Serum aspartate aminotransferase (GOT) activities plotted against ornithine carbamoyl transferase (OCT) activities of 100 alcoholics. Activities expressed in relative values. Numbers indicate how many times the upper normal limit (no. 1) is exceeded.

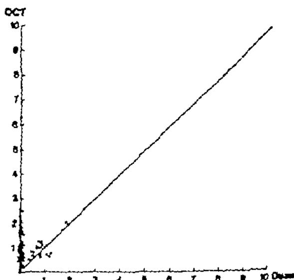


Fig. 6. Serum ornithine carbamoyl transferase (OCT) values plotted against glutamate dehydrogenase (Gdh) values of 100 alcoholics. Other data as in Fig. 5.

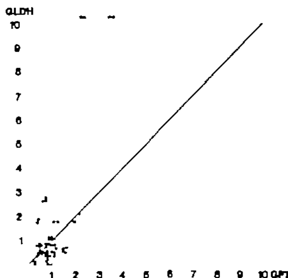


Fig. 7. Serum glutamate dehydrogenase (GLDH) values plotted against alanine aminotransferase (GPT) values as in Fig. 6.

all fourteen patients whose liver extended more than 4 cm below the costal margin, but GOT was normal in four of them.

Guanine deaminase (Guan), called also guanase, is also in clinical practice a quite specific enzyme for liver tissue. Although it is present in large quantities also in the brain and kidneys (63), difficulties arise only seldom in the interpretation of elevated serum guanase deaminase activities. The rather limited clinical experience confirms its value for the detection of liver damage (17 47 53 67). This enzyme test seems, however not to be sensitive enough (67), as was observed also in the present study in which less than half of the cases, with pathological level of OCT showed elevated guanine deaminase values.

Recently γ-glutamyl transpeptidase (γ-GT) has gained increased clinical attention. Due to the organ distribution this enzyme is less specific for liver damage than the two above-mentioned enzymes. γ-GT is present in large amounts in the kidney and less so in the pancreas, spleen, intestine, brain and lung, but only in negligible concentrations in heart and skeletal muscles (31 69 92). In clinical studies serum γ-GT has been found to be increased particularly in obstructive jaundice, but also, although somewhat less, in many other diseases of the liver and biliary tract and in pancreatitis (3 34 36 83, 92). Moderately pathological values have been encountered also in congestive heart failure (83 92) and in myocardial infarction (1 43).

In alcoholics pancreatitis deserves special consideration. In the present study γ-GT was, however normal in three out of the eight cases in which serum amylase was pathological, and in the other cases serum γ-GT was elevated moderately On the other hand serum LAP activity was in good correlation with serum γ-GT activity of the 2_ patients who showed pathological serum LAP activity serum γ-GT activity was pathological in 20 and in the 13 patients who had the greatest increases of γ-GT (exceeding the upper normal limit at least fivefold) all except one had also increased serum LAP activity Serum LAP has been reported to be increased in pancreatic diseases and obstructive jaundice (6, 33 82). It has been shown to reflect cholestasis even more sensitively than alkaline phosphatase (37). Serum LAP seems to be increased in liver diseases less sensitively than serum γ-GT (30 66) which was observed also in the present study In the three alcoholics who had slight hyperbilirubinemia, serum γ-GT activity showed also the greatest elevations, and serum LAP was moderately increased but alkaline phosphatase normal in all of these cases.

Glutamate dehydrogenase (GLDH) seems to have an almost similar specificity for liver tissue as OCT although its activity in the kidneys is about 15% of that of the liver and furthermore an activity of a few per cent of that of the liver is found in most organs (86). In addition GLDH has a special feature of being located intramitochondrially in the cells (9 45). It must thus penetrate two membranes before being able to enter the serum. An increase of GLDH in the serum therefore indicates more severe damage to the cells than, e.g., the increase of serum GPT which is present extramitochondrially in the cells. Pathological changes have been observed in mitochondria of liver cells in alcoholics (74 84 91).

The determination of GLDH/GPT ratio (29) or GLDH/GOT+GPT ratio (86) has been suggested for the differentiation of parenchymal from obstructive jaundice. In the present work serum GLDH was increased somewhat less often than, e.g., OCT but, when increased, the increment was of the same magnitude as, e.g. that of OCT or GOT When the GLDH/GPT ratio

was calculated in the present alcoholics, there was a pattern of "obstructive lesion" in 38 and "parenchymal lesion" in 19% of the cases (Fig. 7). It is questionable, however, whether this ratio is valid, although in all the three cases in which hyperbilirubinemia was encountered the ratio had an obstructive character. There is evidence, however, that the usefulness of these ratios may be questionable in clinical practice. For instance, if the lesion is severe enough in the acute phase of hepatitis, an intramitochondrial fraction of GOT may be released into the serum besides the extramitochondrially located GOT (95).

Creatine kinase (CPK) is characteristically an enzyme of the skeletal and cardiac muscles and of the brain, but not of the liver or other organs (18). Its serum activity very sensitively reflects skeletal muscle (21, 22, 28, 48) but also myocardial (22, 28, 48, 57, 96) damage. In alcoholics an increased serum CPK activity seems to be common. Nygren (68) reported increased values in 38% of alcoholics, which is of the same order as in the present series (43%). An increased serum CPK activity may also be provoked by alcohol ingestion in alcoholics (20). These observations suggest that muscle damage is common in alcoholic patients. This does not necessarily mean that necrotic lesions occur, since mere muscular exercise may evoke an increased serum CPK activity (8), and alcoholics usually have tremor and shivering in the hangover period. In addition alcoholics often show signs of physical trauma. In the present series all seven patients with bruises had an increased serum CPK activity. On the other hand serum CPK is not increased in many other unspecific conditions such as shock (61).

Elevated serum lactate dehydrogenase (LDH) activity in alcoholics seems to be derived either from the liver or from skeletal muscle, but not from the heart, since it was the LDH_5 isoenzyme which was increased, and urea-stable LDH activity showed a lowered activity. Because a similar isoenzyme pattern characterizes both liver and skeletal muscle (97) it is not possible to differentiate these two tissues by the LDH isoenzyme pattern or from urea-stable LDH activity which reflects an isoenzyme-pattern (58, 60, 65).

Muscular exercise may provoke an increase in the serum LDH activity (38), which might possibly indicate skeletal muscles as the origin of increased serum LDH activity in accordance with the observations made with serum CPK. Only in one case did an elevation of serum LDH activity depend on the increased LDH_1 isoenzyme. There was, however, no clinical evidence of myocardial infarction in this patient, and his ECG was normal. Hemolysis as the LDH_1 elevating factor in this patient could not be excluded, although no visible hemolysis was observed, and Zieve's syndrome was not present as judged from serum lipid analyses. The possibility of a pernicious anemia, known to increase LDH_1 activity (25, 70), was not studied, but his hemoglobin was only slightly decreased (12.9 g/100 ml).

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A CASE OF DRUG-INDUCED (?) PULMONARY HYPERTENSION

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Abstract. A case of pulmonary hypertension of primary type with fatal outcome is described. The patient had consumed large amounts of appetite-suppressing drugs. The possibility of causal relationship is discussed.

Primary pulmonary hypertension is an uncommon disease. In the light of recent reports, referred to below about a possible relationship between that disease and the use of appetite-suppressing drugs, we consider the following case to be of interest.

CASE REPORT

Jewish woman, born in 1919 Original domicile Poland, here she was imprisoned during the war in concentration camp, without however contracting lasting bodily injury She came to Sweden in 1945 Three children. No significant earlier diseases.

In Dec. 1967 she became aware of a marked lassitude and, on physical activity sensation of pressure in the precordial region together with breathlessness. Also dull ache in the left shoulder region, and aches and stiffness in the fingers which blanched on exposure to cold. She sought medical attention in Jan. 1968 and was found to have blood pressure of 185/115 No other unusual findings were made. She was given thiazide diuretic and an analgetic. Her condition was unchanged for some time, except that she sporadically had slightly swollen ankles. In April 1968, during travel abroad, she suffered two attacks of severe precordial pain, which was followed by brief fainting. She consulted doctor in Poland and was told that she had heart disease. Digitalis, prednisolone and Rauwolphia preparation ere prescribed. During May and June 1968 she was treated by doctor in her home town. During that period she had marked fording oedema most of the time. No hypertension. X-ray revealed considerable cardiac enlargement She as treated with digitalis and diuretic. On one occasion fainting fit with loss of consciousness during about 10 min occurred. She was increasingly disturbed by fatigue and effort intolerance, becoming severely dyspnoeic even on brief walks indoors.

At the end of July 1968 the patient was admitted to the Department of Medicine, Allmänna Sjukhuset, Malmö, and was treated as an in-patient almost continuously until May 1969 On admission she was noted to have lip cyanosis. Otherwise no signs of cardiopulmonary insufficiency were noted during rest. Cardiac auscultation revealed an accentuated second pulmonic sound with marked fixed split. Blood pressure 150/105 ECG (Fig. 1) showed right axis deviation and right ventricular hypertrophy X-ray (Fig. 2) showed cardiac volume of 700 ml/sq.m body surface, i.e. considerable enlargement, which was predominantly right-sided. An exercise test had to be interrupted after 6 min at 200 kpm/min as the patient was exhausted. Already after 2 min of exercise marked cyanosis was apparent. At rest arterial oxygen tension was 61 mm Hg, i.e. clearly reduced.

Heart catheterization was performed on two occasions, Aug. 21 1968, and Feb. 17 1969 Substantially similar results ere obtained on both occasions. Right ventricular and pulmonary artery pressures were markedly elevated (Table I).

Pulmonary arteriography was performed during both catheterizations. Fig. 3 shows an exposure from the second study There was widening of the pulmonary artery trunk and its two branches, and also to some extent of their primary branches, while the more peripheral vessels were markedly constricted. The picture as interpreted as that of primary pulmonary hypertension.

The patient was treated with digitalis, diuretics (polythiazide, later furosemide) and dicumarol. The clinical impression was that slow deterioration was taking place, effort dyspnoea becoming increasingly prominent. As rule she was obviously cyanotic even in the resting state. There ere no new features in the disease picture, except that in Sept. 1968 she started to complain of dull pain in the left abdominal flank. Palpation gave the impression of mass in that region. Intravenous urography revealed that both kidneys were moderately enlarged, with deformation of the intrarenal excretory

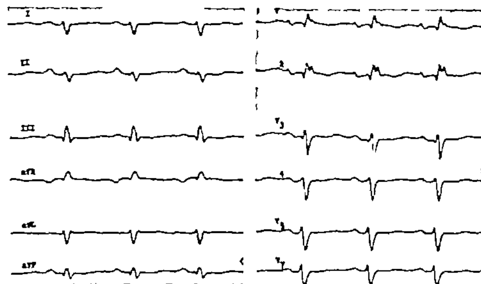


Fig 1 Regular sinus rhythm, right axis deviation, incomplete right bundle branch block, and signs of right ventricular hypertrophy

suggestive of polycystic kidneys. Serum creatinine was intermittently slightly elevated, but endogenous creatinine clearance gave a normal value. There was an intermittent microscopic haematuria.

During Nov and Dec, 1968 there were increasing signs of right ventricular failure. A combination of de and spironolactone had a fairly good effect, complete eradication of dependent oedema not obtained. During this period the patient was almost totally immobilized with constant dyspnoea hich abated somewhat on the administration of oxygen. She also had sensation of cold in hands and feet. Signs of impaired peripheral circulation were noted, the radial pulses were extremely faint, and in the legs there re no palpable pulses distal to the popliteal artery. Systolic blood pressure had for some time showed decreasing tendency and came down to a tourniquet pressure of 110–120/95 mm Hg. Venous occlusion plethysmography



Fig 2. Thoracic X-ray showing cardiomegaly predominantly right-sided.

Table I. *Data from heart catheterizations*

PCV – pulmonary capillary venous pressure (indirect measure of left atrial pressure)
PVR – pulmonary vascular resistance

	Aug. 21 1968	Feb. 17 1969	Normal
PCV mm Hg	2	15	2 12
Mean pressure pulmonary artery mm Hg	56	65	<25
Right ventricular pressure, mm Hg	90/3–9	100/3–13	35/0–10
Right atrial pressure, mm Hg	5	14	<5
PVR, dyne-sec cm^{-5}	1341	1420	<200
Cardiac output, l per min (Fick)	3.2	2 8	5 0
Arterial pressure, mm Hg (puncture)	*146/110*	*122/97*	
Lung function tests	Essentially normal	Not done	

Interpretation of above findings: Pronounced elevation of pressure in pulmonary circulation with essentially normal left atrial pressure. These findings together with the very marked increase of PVR and the practically normal spirometric data are compatible with the diagnosis of primary pulmonary hypertension.

on the legs showed severely reduced blood flow On few occasions there were small, sharply punched out necrotic lesions in the skin of the legs. These were considered to be of vascular origin (epidermal infarctions). There were no ischaemic changes in fingers or toes.

From the beginning of Jan. 1969 the patient was treated with continuous oxygen administration through nasal catheter This treatment was tried because of reports in the literature (1) that long-term oxygen administration may result in lowering of the pulmonary artery pressure in cases of pulmonary hypertension caused by primary pulmonary disease. Naturally the applicability of this in the present patient was dubious. During oxygen administration the arterial oxygen tension was normal or higher than normal. The problem of fluid retention remained: there was considerable oedema of the legs and during one period ascites. The diuretic therapy consisted of furosemide plus spironolactone plus salt-restricted diet and, in addition, occasional injections of mercurial diuretic.

A clinical impression that some improvement took place during the oxygen treatment was not verified at the second heart catheterization (Table I). Thoracic X ray and pulmonary arteriography likewise showed no change. No similar studies were made, however, during the period (Dec. 1968) when the patient' general condition was at its worst, and thus it was considered possible that some improvement had occurred since then.

During March and April 1969 the patient was given oxygen only intermittently She felt less dependent on the oxygen than during the start of the treatment period. Objectively she was in somewhat better condition, being free of oedema most of the time. On cautious bicycle ergometer training an increasing exercise tolerance was noted.

The patient was discharged at the beginning of May 1969 During the following three months her condition was stationary and relatively satisfactory She as equipped with oxygen in her home but used it infrequently She could move about indoors fairly freely and was also able to take small walks in the garden, although always disturbed by sensation of breathlessness and fatigue when moving.



Fig 3 Pulmonary arteriography widening of pulmonary artery and its main branches, bereas peripheral arteries are distinctly narrowed.

induced by that preparation (including a fatal case in a Danish hospital where the drug had been made available on trial) (5 6, 7 9 10 12, 13). In addition there have been reports from Germany about four cases where the PH was suspected to be causally related to the anorectics, chlorphentermine and "kloforex (the ethyl ester of the carbamic acid derivative of chlorphenter mine) (6). Also as could be expected a number of patients with PH have used both aminorex and other appetite-reducing drugs, such as phen metrazine (10, 13) and phentermine (13) one patient had taken only amphetamine, although several years before the onset of symptoms (10)

In the cases reported upon, the clinical picture has been rather uniform. The patients are, with few exceptions, female, middle-aged, of previously good health, moderately overweight or normal weight. A few months after the start of aminorex medication there has been a gradual onset of effort dyspnoea, fatigue and ankle oedema. In the course of several months deterioration to a state of fully developed right ventricular failure has occurred, the main symptoms being anginal pains, oedema, cyanosis, in some cases dyspnoea at rest, fainting and haemoptysis. Physical examination has revealed strongly accentuated second pulmonic heart sound and increased right ventricular pulsations. ECG has shown, in the majority of cases, a pulmonic P wave, a right axis deviation, and signs of right ventricular hypertrophy X-ray of the thorax has shown an enlarged right heart and a prominent pulmonary artery with penguin hilus" pattern. Lung function studies have not indicated primary pulmonary disease. Haematological and biochemical studies have revealed nothing of interest except occasional slight polycythaemia.

Twenty-eight cases have been described in detail (10 12, 13) and the following data are quoted. twenty-five were women, average age 41 years. Onset of symptoms occurred on the average nine (1–30) months after the start of aminorex medication. Cardiac catheterization revealed a precapillary PH with a mean pressure in the pulmonary artery of 51 (33–69) mm Hg, a pulmonary capillary venous pressure (PCV) of 8 (2–14) mm Hg and a pulmonary vascular resistance (PVR) averaging 1140 (450–1960 normal less than 200) dyne-sec cm^{-5} Three patients died. Necropsy in one of these (12) showed primary pulmonary arterial sclerosis without signs of thrombo-embolism. A similar picture of hyper plastic and fibrosing arterial changes was found in four lung biopsies (10).

The temporal coincidence between the use of aminorex and the occurrence of PH of primary type has invited speculation about a possible pathogenesis. Fatty micro-embolism, thromboembolism and hypoventilation caused by a central disturbance have been suggested, but have hardly found any support in the data available The most obvious explanation would be that the drug causes a direct vasoconstriction by stimulating adrenergic alpha receptors in the smaller pulmonary arteries. A protracted vascular spasm is believed to be capable of causing in blood vessel walls the changes of medial hypertrophy intimal proliferation, and fibrosis, i.e. the kind of picture which has been seen in the patients mentioned and which is also characteristic of primary pulmonary hypertension without any known or suspected causative factor

Aminorex, like other anorexiant drugs, is related to amphetamine as well as to the typical alpha receptor stimulants, i.e noradrenaline and others (Fig. 7)· thus a vasoconstrictor action would not be surprising. However in symptom-free users of aminorex no elevation of pressures in the lesser circulation has been found (13). Furthermore, there is a lack of an explanation of the absence of systemic hypertension in the patients. Finally the number of people falling ill is only a minimal proportion of the users of aminorex, which have been calculated to be 30,000 in Austria alone (in all three countries mentioned the drug has been released for sale without prescription and has gained a dominant share of the anorexiant market, probably because of a noticeable euphoriant action). Those individuals who contract PH would therefore seem to be in some way perhaps genetically predisposed.

An additional enigma is the question why aminorex should be the only drug in this class to manifest this suspected side effect, consider ing that all anorexiant drugs are closely related, insofar as all of them are derivatives of the phenylethylamine nucleus of amphetamine. Aminorex, though, differs in its cyclic side-chain. In countries where aminorex has not been introduced, (Scandinavia, Poland, Czechoslovakia,

Great Britain, France and USA, among others) no increase in the incidence of primary PH seems to have occurred.

Of the other drugs mentioned above, chlorphentermine (Lucofen®) and its long-active derivative "kloforex" (Oberex®) have been marketed in Sweden but were withdrawn in March 1969. The closely related compound phentermine (Mirapront®) is still available.

Regarding the patient described here the consumption of appetite-suppressing drugs is large enough to be striking per se and, since the disease she contracted is rare, we felt that a certain suspicion of a causal relationship should be entertained in the light of the aminorex cases. It should be emphasized that the patient's history and the detailed laboratory work-up as well as the necropsy failed to provide any evidence of other conditions that could have produced the PH, such as pulmonary parenchymal disease, thrombo-embolic disease, generalised arteritis, collagen disease, or cryoproteinaemia. The patient's drug consumption is dominated by diethylpropion, which is available in Sweden under five proprietary names, the tablets consisting of the pure drug or of a combination of it with a sedative and/or an anticholinergic. Diethylpropion has not been indicated as a suspected inducer of PH, but in the present case it could conceivably have been of importance. Whether the patient had at all used kloforex could not be elucidated with any certainty; if used, it was in a small quantity during a brief period when her symptoms started to develop. Her use of phentermine was brief and was not started until after definite symptoms had already developed. The patient had taken d-amphetamine, although several years ago; it should be noted that it was widely used in several countries, including Scandinavia, in the forties when it was sold without prescription, no unusual incidence of PH seems to have resulted during that period.

The presence in this case of polycystic kidneys is most probably a chance coincidence. The combination of polycystic kidneys and pulmonary hypertension has not been described in the literature on these diseases. Morphologically the kidneys were not felt to be typical of hereditary polycystic disease but rather looked upon as an unusually marked accumulation of retention cysts. Since virtually all of the patient's relatives were killed during the war a family history could not be obtained.

Space does not permit a detailed scrutiny of the reasons for and against the assumption that aminorex may cause the development of pulmonary hypertension. Those suspicions that have been publicized are based practically exclusively on statistical considerations. The situation was initially the same in the thalidomide disaster in which, however, the number of cases was so large that a causal relationship emerged as almost proven as soon as the suspicion had been raised. With the present drug the numbers involved are much smaller and the conclusions correspondingly less secure. In a few of the reported cases an improvement in the patients' condition has been noted after the aminorex administration was interrupted. Furthermore, there now exists in Germany, Austria and Switzerland a possible opportunity to secure a cause-and-effect relationship by finding a decreasing incidence of primary PH after the retraction of aminorex from the market.

Concerning the patient reported here, it is self-evident that the idea of a causal relationship between the drugs and the disease is of a hypothetical nature, but further observations with this group of drugs may be warranted.

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SUCCINIC DEHYDROGENASE ACTIVITY IN SKELETAL MUSCLE OF NORMALS AND PATIENTS WITH DYSTROPHIA MYOTONICA

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Abstract. Succinic dehydrogenase activity in skeletal muscle, oxygen consumption at rest and body cell mass have been examined in a group of patients with dystrophia myotonica. For comparison a group of middle-aged blood donors and young persons before and after physical training were included, as well as a middle-aged well-trained group. The enzyme activity was on an average not lower in the dystrophia myotonica group than in other untrained groups. Body cell mass decreased, however, with decreasing physical activity in dystrophia myotonica. Oxygen consumption was also low. It was concluded that it is not likely that dystrophia myotonica has a deficiency in the enzymatic aerobic capacity as a primary defect. A low muscle mass with a normal aerobic capacity might contribute to the well-known decrease of oxygen uptake in these patients.

Dystrophia myotonica (DM) is characterized by symptoms from several organs. The most important are the symptoms from skeletal muscle, with the myotonic phenomenon and atrophies starting in the periphery. One feature of the disease is a decrease of basal oxygen consumption. This is apparently not due to a decrease in thyroid function. It might therefore be caused by a deficiency of the peripheral equipment for oxygen uptake in a quantitative and/or qualitative way.

The present work was undertaken to study this problem. Skeletal muscle was chosen for study because the symptoms of the disease mainly come from this tissue. Furthermore, skeletal muscle constitutes the major single tissue with high capacity for oxygen consumption, and derangements of mitochondrial structure have been observed in DM (1, 5, 7). The capacity of muscle cell for oxygen consumption was estimated by determination of succinic dehydrogenase, an enzyme closely attached to the mitochondrial structure (8, 12). In order to get an approximation of muscle mass, total exchangeable potassium was also determined.

MATERIAL

Eleven patients with DM were selected for studies. They all had findings typical of DM such as muscle atrophy and active or mechanical myotonic reactions and typical findings in electromyographic investigations. Six patients had myotonic cataract. Seven of the patients were women, 30–62 years, and four men, 42–69 years of age.

The physical abilities of the patients were graded into three groups. Group I had no impairment of their physical activities. Group II had a clear decrease in different activities but were still able to take care of themselves without assistance. Group III included patients who were totally disabled and sitting in a wheelchair. These function groups included two, seven and two patients, respectively.

Thyroid function was investigated repeatedly in the patient group with clinical routine determinations of protein-bound iodine and triiodothyronine in blood, turnover of radioactive iodine and serum cholesterol, and all were normal in this respect. Two patients (nos. 7 and 10) had been treated with *l*-thyroxin (0.1 mg daily) for several years.

As control served a group of six randomly selected persons, operated on for varicose veins under general anaesthesia with barbiturates (Pentobarbital, Evipan from Bayer, Leverkusen, Germany), nitrous oxide and succinyl choline. Furthermore, a second group of ten blood donors, men aged 50–56 years, were included. These probably had a varying degree of everyday physical activity but were not engaged in athletic activities. A third group consisted of seven men, aged 36–60, mean 51 years, who were very well trained and had been active competitors in cross-country running or skiing for decades. A fourth group consisted of seven medical students, aged 20–23 years, two women and five men, who were

Table I. *Data of patients with dystrophia myotonica*

Pat. no.	Sex	Age (y)	Function group	Body weight (kg)	Body cell mass (kg)	Oxygen uptake (l/min)	Succinic dehydrogenase activity (μl O_2/g wet weight muscle/h)	Succinic dehydrogenase activity (μl O_2/mg muscle protein/h)
1	♂	42	I	64	22.5	0.21	1962	25.9
2	♂	54	II	70	18.2	0.25	1482	10.9
3	♂	65	II	65	18.6	0.19	2131	31.1
4	♂	69	III	63	11.6	0.20	1129	8.5
5	♀	30	I	55	18.9	0.18	2413	—
6	♀	45	II	47	12.8	0.13	2081	34.2
7	♀	51	II	82	15.6	0.18	1713	5.6
8	♀	57	II	63	13.4	0.17	2186	27.3
9	♀	62	II	70	15.3	0.19	3195	—
10	♀	52	II	85	18.9	0.25	2228	—
11	♀	50	III	59	11.2	0.18	808	20.2
	Mean ± S.E.M.			65.7 ± 3.3	16.1 ± 1.1	0.19 ± 0.01	1957 ± 199	20.5 ± 3.9

physically untrained but subjected to a controlled training program (15). They were tested before and after this training period.

METHODS

Muscle biopsy was taken for determination of succinic dehydrogenase activity from the lateral vastus muscle on the middle of the thigh of the DM patients and the controls. In nine of the DM patients this was performed local anaesthesia (Carbocain from Bofors, Mölndal, Sweden). In the other groups and in two of the DM patients percutaneous needle biopsy technique (2) was used. In the untrained middle-aged group the biopsy was repeated once, about two weeks after the first biopsy. These biopsies were also performed under local anaesthesia. In all DM patients at least triplicate determinations of the enzyme were performed in different parts of the muscle sample.

The muscle samples were put into ice-cold 0.25 M sucrose solution and brought to the laboratory where they were freed from visible fibrous tissue, fat and other non-muscle tissue, blotted and weighed on a torsion balance. Thereafter they were homogenized in 0.25 M sucrose and parts of the homogenate were taken for succinic dehydrogenase determinations. Part of the homogenate was also taken for protein measurements according to the method of Lowry et al. (11). Succinic dehydrogenase was then assayed manometrically as previously described (3).

Total exchangeable potassium was determined in the DM patients according to the method of Lindholm (9, 10). Body cell mass was obtained as described by Moore et al. (13). Oxygen consumption was measured in a Krogh spirometer at rest in the morning without previous physical activities.

In two cases (nos. 2 and 4) a biopsy of musculus pectoralis major was taken under light anaesthesia with barbiturates (Evipan, Bayer). This muscle sample was homogenized and mitochondria isolated by fractional centrifugation. Oxygen uptake with glutamate or pyruvate-malate as substrate, respiratory control and adenosinetriphosphatase activity before and after 2,4-dinitrophenol stimulation were measured. These methods have been described previously (4). The biopsy from patient 2 contained so much fibrous material that isolation of mitochondria was not possible.

RESULTS

Table I summarizes the results of measurements in the DM patients. It is seen that body cell mass roughly follows the degree of physical function in the patients. Oxygen uptake was 10% or more below predicted normal values taken from routine standards at appropriate body surface, age and sex.

The muscles with very low activity were noticed to be macroscopically much lighter red than those muscles with a high activity which were dark red.

Fig. 1 gives the results of enzyme determinations in the different groups. When expressed per gram wet weight the subjects with varicose veins, untrained middle-aged blood donors, and untrained young medical students and the DM patients showed no difference between their values. Trained middle-aged men showed a trend ($p < 0.10 > 0.05$) to higher values than the untrained. The values of trained medical students were higher than before training ($p < 0.01$). When expressed per unit muscle protein, subjects with varicose veins showed a trend ($p < 0.10 > 0.05$)

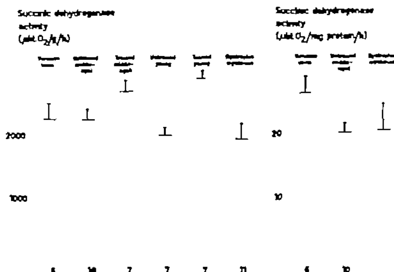


Fig 1 Succinic dehydrogenase activity in the vastus lateralis muscle of different groups of subjects. Left part of figure gives values in relation to muscle weight, in right part in relation to muscle protein Means and S.E.M.

to higher values than untrained middle-aged men, otherwise there were no differences.

The isolated mitochondria from patient 4 showed a respiration with glutamate and pyruvate-malate of, respectively 4.40 and 4.23 µatoms of oxygen taken up per mg protein per 20 min. The respiratory control ratios for these two substrates were, respectively infinity and 2.7 and adenosinetriphosphatase activity 1.81 micromoles of inorganic phosphorus per mg mitochondrial protein per 20 min before, and 4.31 after 2,4-dinitrophenol activation. All these values are within the range of those found in normal patients (4).

DISCUSSION

Recent work suggests that physical training in the rat increases the potential of skeletal muscle to take up oxygen by increasing the activity of several enzymes taking part in aerobic metabolism (6) This seems also to be possible in at least young, healthy persons after fairly limited time f hard physical training (15). The present data suggest that this is also possible in middle-aged men, but has to be tested in the same group of subjects before and after training before definite conclusions are possible.

The subjects operated on for varicose veins had a low every-day physical activity and were included to control the factors introduced into the study by surgical intervention and general anaesthesia. These patients and the two groups of untrained subjects had similar values of succinic dehydrogenase. The DM patients did not differ from these untrained groups when expressed per unit weight of muscle. When protein was the basis for expression of activity the subjects with varicose veins showed a trend to higher values for a reason which is unknown.

One might consider that a deficient oxygen uptake system in the cells of tissues giving symptoms in DM could be a primary deficiency in this disease. Of these tissues the succinic dehydrogenase activity of muscle was investigated but did not show any differences in DM patients as compared with controls. Therefore, a deficiency of the oxygen uptake system is probably not the primary etiological factor in this disease.

The low oxygen uptake in relation to the predicted normal value in patients with DM might be caused by qualitative or quantitative changes in the enzymes for aerobic metabolism. A qualitative deficiency in the aerobic system is thus not likely because the low oxygen uptake values at rest were in some DM patients combined with a rather high succinic dehydrogenase activity (cf Table I) Furthermore, oxidative phosphorylation was normal in proximal muscle of one patient with advanced DM.

There is, however also quantitative aspect of this problem. A normal aerobic capacity per unit muscle would in a small total muscle mass produce a total low oxygen uptake. Therefore

total exchangeable potassium was determined in order to quantitize body cell mass, of which muscle mass is a considerable part (13). The body cell mass in DM patients with a body weight of 67.5 kg was 16 1 kg compared with a randomly selected population of middle-aged men in whom the body cell mass was 31 kg and body weight 76 kg (14)

It therefore seems likely that the decreased muscle mass in advanced cases with this disease should contribute to a decreased capacity to consume oxygen, simply because metabolically active tissue has disappeared, since no decrease in enzymatic capacity to consume oxygen could be demonstrated.

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POISONING WITH ETHYLENE GLYCOL MONOMETHYL ETHER

Report of Two Cases

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Abstract. The clinical and laboratory features of two patients with ethylene glycol monomethyl ether poisoning are presented. By accident, both patients concomitantly had ingested about 100 ml of the toxic agent. After a symptom-free interval of 8–18 hours, they became mentally confused and complained of general weakness and nausea. In addition, deep and frequent respiration together with profound metabolic acidosis was found. One of the patients, in whom marked oxaluria as demonstrated, initially had transitory rise in the serum creatinine level. Both cases made an uneventful recovery. The similarities between the clinical picture described and that of poisoning with pure ethylene glycol and also methanol are emphasized and might support the assumption that ethylene glycol monomethyl ether is hydrolysed in man ith formation of the two former compounds.

Ethylene glycol monomethyl ether (CH_3OCH_2-CH_2OH) is a volatile almost odourless liquid with bitter taste. It has been commercially available for several years, and is used industrially as solvent, detergent, as moistening and drying agent and as non-corrosive anti-freeze preparation.

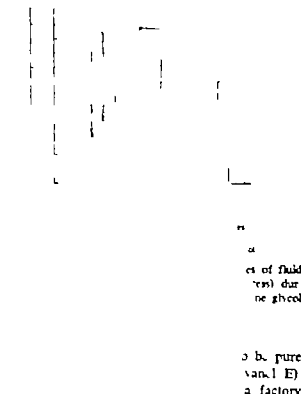
The toxicity of the agent has been extensively studied in animals (5, 14, 15). According to these authors, inhalation or parenteral administration of the material may cause systemic toxicity mainly affecting the kidney (hematuria, albuminuria, urinary casts), and to a lesser extent the liver and the brain. In man regular inhalation of a moderate concentration of ethylene glycol monomethyl ether may cause "toxic encephalopathy" with weakness, headache, disorientation, and in many cases psychiatric signs and mental retardation have been observed (1, 4, 9). The literature on the toxicity after peroral ingestion is more scanty and the only case found to be published is that recorded by Young and Woolner (16). Their patient was admitted to hospital in a comatose condition, and he died 5 hours later without regaining conciousness. The main autopsy findings were edema of the brain, marked fatty degeneration of the liver and microscopic evidence of degenerative and toxic changes in the renal tubules. The metabolic breakdown of the ether in the human organism is also unclear. Chemical considerations might suggest that the agent is hydrolysed with the formation of methanol and ethylene glycol. Animal studies, however, have failed to demonstrate any increase in methanol or formic acid in the urine, or in the excretion of oxalic acid which is the oxidation product of ethylene glycol (15). In the case described by Young and Woolner the urine contained no methanol, thus supporting the assumption that the ether is not hydrolysed in the human organism.

The present two cases with peroral poisoning with ethylene glycol monomethyl ether are reported for the following reasons:

1. The cases presented a clinical picture of interest to internal medicine as well as to industrial toxicology.

2. Some f the metabolic observations made were informative for the study of the breakdown f the ether in man.

Two males consumed together on Sep. 15 1968, about 100 ml each of a fluid supposed to be common alcohol. Some hours afterwards they were taken ill and admitted to the hospital. Here they presented a clinical picture interpreted as ethylene glycol poisoning. They were therefore treated with sodium bicarbonate and ethyl alcohol intravenously by drip for three days (11).



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20 hours after ingestion of the toxic agent.

On admission he w p r sodium. The predominant clinical features b de mental disturbances were marked muscular weakness, vertigo and nausea. The respiration as frequent and deep. The blood pressure w 150/100 mm H pulse 115 min, regular temperature 37.0°C. Except for dry rales heard at the base of both lungs, the physical examination wa unremarkable. The urine was acid with specific gr ity of 1020. There was marked proteinuria. On microscopical examination hite blood cells and oxalate crystals were found. Hb 16.4 g/100 ml, ESR mm h, W B C 1 600 mm³ serum creatinine ..0 mg/100 ml, serum N 140 mEq/l, serum K 4.5 mEq/l, serum Cl 109 mEq l, serum C 4.7 mEq/l. The arterial blood pH was 7.18 pCO₂ was 17.5 mm Hg, the total CO_2 content 6.8 mEq l, and the base excess − 1 mEq/l.

On the first day he was treated with intravenous infusion of 2000 ml sodium bicarbonate 4.2% and 750 ml of 5% ethyl alcohol in glucose, infused at an estimated rate of 100 ml/h. On the following two days he was given 2500 ml and 2000 ml, respectively, of 5% ethyl alcohol, in a solution containing glucose with K⁺ 12 mEq/l, and Na⁺ 52 mEq/l added. He also received glucose intravenously and was forced fluid. The total fluid intake on the first three days after admission varied between 3.5 and 5.. l. The urinary output showed a tendency to decrease, but increased again after administration of mannitol, and an adequate diuresis was maintained. On this regimen a rapid correction of the acid base disturbances was obtained (Fig. 1).

Clinically he improved more slowly. The serum creatinine reached normal level on the 5th day, while a moderate proteinuria persisted for one week. During the same period the patient was hypokalemic (lowest serum K ..2 mEq/l), as well as hypocalcemic (lowest serum Ca 3.6 mEq/l). The urinary oxalate content was determined during the first week after admission. The amount of oxalic acid excreted varied between 10 and 1000 mg/24 h. No methanol or ethylene glycol monomethyl ether was found in the urine. There were normal findings by ophthalmological examination.

The course was complicated by a bronchopneumonia. Transitorily he had a partial loss of hair and he developed dermatitis. Otherwise he made slow but uneventful recovery and was discharged 4 weeks after admission.

Case

The patient was a 23-year-old man who was admitted to Medical Department VII as an emergency case on the same day as case 1. About 18 hours after ingestion of the toxic agent, while walking to his office, he rapidly developed progressive muscular weakness. On admission a few hours later he presented clinical features which were similar to those described in case 1, but not so pronounced. He was moderately confused. The respiration was deep and frequent. The blood pressure was 140/110 mm Hg, pulse 120/min, regular, temperature 39.0°C.

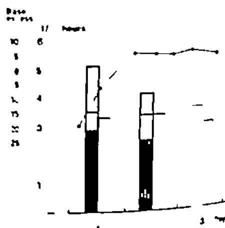


Fig. Clinical course of case .. Legend as in Fig

Physical examination showed no abnormalities. The urine was acid with specific gravity of 1018. There was moderate proteinuria. Microscopically few white blood cells were found. No oxalate crystals were present. Hb 16.4 g/100 ml, ESR 1 mm/h, W.B.C. 19.000/mm³ serum creatinine 1.5 mg/100 ml, serum N 147 mEq/l, serum K 4.8 mEq/l, serum Cl 109 mEq/l, serum Ca 5.0 mEq/l. The arterial blood pH was 7.22, pCO_2 was 18.5 mm Hg, the total CO_2 content 7.8 mEq/l and the base excess −19.0 mEq/l.

During the first three days after admission the patient received the same therapeutic regimen as described for case 1. The acid: base disturbances were corrected within the first 4 hours. An adequate diuresis was maintained (Fig. 2). The serum creatinine was normal after the first day. A slight proteinuria persisted for one week. There were no abnormalities in the serum electrolyte levels. The daily amount of oxalic acid excreted in the urine was determined from the 5th to 9th day after admission and varied between 27 mg and 54 mg/24 h. No oxalate crystals were found. There was no methanol or ethylene glycol monomethyl ether in the urine. No biochemical evidence of liver damage was found. There were normal findings by ophthalmological examination.

The patient was discharged three weeks after admission.

DISCUSSION

The two cases demonstrated that single-dose oral ingestion of 100 ml ethylene glycol monomethyl ether may cause serious toxic reactions in man. The main symptoms were general weakness, disorientation, muscular restlessness, nausea and vomiting. The clinical symptoms appeared from 8 to 18 hours after the ingestion. The essential clinical features were cerebral confusion, a deep and frequent ventilation and a profound metabolic acidosis. A moderate renal failure was observed initially in one of the cases, together with a marked oxaluria persisting for at least one week. The patients made an uneventful recovery although the general condition improved slowly.

The mental disturbances with cerebral confusion which developed several hours after ingestion resembled the "toxic encephalopathy" described in cases regularly exposed to the vapours of ethylene glycol monomethyl ether (1, 4, 9). The pronounced hyperventilation was also present in the case of acute peroral ethylene glycol monomethyl ether poisoning published by Young and Woolner (16).

The development of a profound metabolic acidosis is relevant to the discussion of the metabolic breakdown of ethylene glycol monomethyl ether. The acidosis might suggest a hydrolysis of the ether to methanol and ethylene glycol which are metabolized to formic acid and oxalic acid respectively. The assumption that such a hydrolysis occurred is supported by the observation in one patient of an increased urinary excretion of oxalic acid. Normally the daily amount of oxalate excreted in the urine is 10–40 mg. In case 1 the urine contained up to 1000 mg per day during the first week. The rather pronounced oxaluria in this case does not seem to have been demonstrated in animal experiments. However, similar findings have been reported following ethylene glycol poisoning by Flanagan and Libcke (3). In their case numerous oxalate crystals were found in the urine and in the renal parenchyma, but no quantitative estimation of urinary excretion of oxalic acid was made. Their patient, who survived, developed acute renal insufficiency during the initial stage of the poisoning and was treated with peritoneal dialysis. The absence of any significant renal failure in our case is not in accordance with the findings in oxaluria secondary to acute ethylene glycol poisoning or in cases of idiopathic familial oxalosis (2, 7).

The suspicion of pure ethylene glycol poisoning in the initial phase served as the basis for the administration of ethylene alcohol in addition to the traditional treatment of metabolic acidosis. In vitro experiments have shown that ethyl alcohol may prevent the oxidation of methanol as well as ethylene glycol, if given in sufficient concentrations to compete for the active sites of the human liver enzyme alcohol dehydrogenase (12, 13). The benefit of ethyl alcohol administration in methanol poisoning is well known (8, 10). Ethanol was proposed in ethylene glycol poisoning by Wacker et al. (11), who found that the oxaluria promptly disappeared after the beginning of ethanol therapy. The use of ethyl alcohol in the initial treatment of ethylene glycol monomethyl ether poisoning was thus most probably of value for the successful outcome in the present cases.

The remarkably high urinary output of oxalic acid observed in case 1 shows that some of the ethylene glycol apparently had been oxidised in spite of the ethanol given. The present study gives no explanation of this observation. However, in addition to the liver alcohol dehydro-

genase the oxidation of ethylene glycol also depends on the catalase activity From the in vitro experiments published by Keilin and Hartree (6) it is possible that the aerobic oxidation of ethanol to acetic acid generates H_2O_2 which stimulates the oxidation of ethylene glycol via catalase. Theoretically ethanol administration might enhance the catalase-mediated oxidation of ethylene glycol, thus explaining the large excretion of oxalic acid observed in one of the patients.

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HEMOLYSIS DURING ACUTE EXERCISE IN PATIENTS WITH AORTIC BALL VALVE PROSTHESES

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Abstract. Physical exercise may provoke increased erythrocyte destruction in patients with severe hemolytic anemia following the insertion of intracardiac prosthetic materials. The relationship between physical exercise and intravascular hemolysis has, however previously not been studied in non-anemic patients with properly functioning ball-valve prostheses or in unoperated patients with valvular disease. Therefore, intravascular hemolysis was studied before and after graded exercise test of 15 min duration in three groups of patients: 16 cases with aortic ball valves, 13 cases with unoperated aortic valvular disease and a control group of ten subjects. Serial measurements of plasma heme concentration and serum lactic dehydrogenase activity (SLDH) were made during two-hour period. Hemodynamic parameters were recorded during the exercise. All patients with ball valve prostheses showed an increase of SLDH averaging 33 U/l, corresponding to destruction of red cells in about 20 ml of whole blood during the exercise. Hemolysis of this order of magnitude was not detected by plasma heme measurements, but the average heme concentration was significantly elevated in the patients with ball valves. In controls and unoperated patients neither plasma heme nor SLDH changed consistently following exercise. The discrepancy between the changes in plasma heme and SLDH is discussed, red cell destruction by the ball valves is the most probable cause of the SLDH increment. There was no significant correlation between the SLDH increase during exercise and the ball valve size, heart rate, cardiac *output or the SLDH level* in the resting state. Probably individual factors are more important determinants of hemolysis than hemodynamic parameters.

Chronic intravascular hemolysis caused by foreign materials inserted into the heart is well known (2, 10, 16) About 80% of patients with Starr-Edwards aortic ball valves exhibit signs of hemolysis (10). In most cases the increased red cell destruction is easily compensated for by the bone marrow therefore significant hemolytic anemia is observed in only bout 5% of the patients (13). In these few patients, however hemolysis may be an important determinant of prognosis, and may indicate both paravalvular insufficiency and ball variance (8). Intravascular hemolysis has also been demonstrated in patients with unoperated disease of the heart valves (2, 10) and main vessels (12). Although the mechanisms is not clarified in all details, most authors believe that turbulence and direct contact between red cells and solid surfaces are the most important causes. We have suggested that mechanical stress in the peripheral arteries may be an additional factor in aortic insufficiency (10).

In two patients with intracardiac prosthetic materials Sears and Crosby (14) observed that the hemolysis showed marked diurnal fluctuations related to physical activity This has been confirmed by others demonstration of diurnal variations in urinary iron excretion (7 16), probably reflecting increased hemolysis during periods with increased cardiac output. However these reports deal with few cases with frank hemolytic anemia. Exercise-induced hemolysis has not been demonstrated in ordinary non-anemic patients with properly functioning ball valves or in unoperated patients with valvular disease. The aim of this study was to evaluate the frequency the degree and the causes of exercise-induced hemolysis in such patients.

MATERIAL AND METHODS

Three groups of subjects were studied. *Group I* consisted of seven healthy volunteers and three patients with non-circulatory disorders. *Group II* consisted of thirteen unoperated patients with severe aortic valvular disease. None of them have later been operated upon, and two have been re-examined and included also in group III. Four patients suffered from pure aortic stenosis with peak systolic gradients across the valves exceeding 100

Table I. *Variations in plasma heme concentration (mg/100 ml) after exercise*

			Plasma heme concentratio						
		N of		After exercise, min					
Group	No. of cases	estimations	Before exercise	0	15	30	60	120	Mean ± S.D.
I. Normals	20	64							5.1 ± 2.7
II. Unoperated aortic valvular disease	13	142	5.7	5.8	6.3	5.5	5.7	5.0	5.6 ± 2.0
III. Aortic ball valve prosthesis	16	164	7.5	8.3	7.4	7.9	8.1	7.5	7.8 ± 3.3

The difference between the means of group III and the other groups are statistically significant ($p < 0.01$). The other differences are non-significant.

mm Hg, five had a pure aortic regurgitation, and four combined aortic stenosis and insufficiency. All cases were examined by left heart catheterization, coronary arteriography and, in most cases, left ventricular angiography. *Group III* consisted of fourteen patients in whom an aortic silastic ball valve prosthesis had been inserted five to twelve months previously. Two patients had Magovern valves, the others Starr-Edwards valves of various size. In three patients a mitral commissurotomy was performed during the same operation. One patient developed a complete atrioventricular block, and a permanent fixed rate pacemaker was implanted. In all cases the operation was clinically evaluated as successful. Several patients still complained of reduced effort tolerance and various other symptoms at the time of the study. In none of them have auscultatory or other signs of paravalvular leakage been observed. Eleven cases from group III showed ahaptoglobinaemia, the remaining three had haptoglobin values below 15 mg/100 ml. No patient was in congestive heart failure at the time of the study and there were no signs of liver congestion. Anemia was not present in any of the subjects examined.

All subjects were kept in bed for the last 16 hours prior to the examination. Serial measurements of serum enzymes and total heme plasma pigments were made before, immediately after, and 15, 30, 60 and 120 min after a graded bicycle ergometer exercise test of 15 min duration. Cardiac output and heart rate were registered before and during the exercise in all the patients. In the normal subjects only heart rate was recorded. The work load was graded according to subjective symptoms and heart rate; no one was forced to accept a discomfortable load. The maximally allowed heart rate was 150 beats/min, and the maximal load applied 600 kpm/min. Peripheral muscular strength seemed to be a more limiting factor than heart rate and dyspnea in several patients, especially in group III. The mean terminal load in the three groups was 600, 500 and 450 kpm/min, respectively. No complications followed from the procedure, except for a transitory fall in blood pressure after the test in one patient from group III. In no case was anginal chest pain provoked.

Cardiac output was measured by the dye-dilution technique using indocyanine green as indicator. According to our experience this did not in itself give rise to errors in plasma heme or enzyme measurements.

Total plasma heme pigments were determined by the benzidine method of Crosby and Furth (5). Blood sampling was made as nontraumatic as possible, in most cases through in-dwelling catheters, using no or very small suction pressure. The blood was carefully let into heparinized plastic tubes and immediately centrifugated in

Table II. *Variations in SLDH (U/l) after exercise*

			SLDH (U/l) ± S.D		
			After exercise min		
Group	No. of cases	Before exercise	0	30	120
I. Normals	10	143 ± 30	137 ± 31	137 ± 27	132 ± 23
II. Unoperated aortic valvular disease	13	143 ± 29	150 ± 38	142 ± 28	136 ± 32
III. Aortic ball valve prosthesis	14	256 ± 88	289 ± 98	284 ± 103	292 ± 105

The differences between the values in group III and values in groups I and II are statistically significant ($p < 0.01$) at all times. The difference between the values before and at all times after exercise is statistically significant ($p < 0.01$) in group III. The other differences are non-significant.

two steps, one at 300 *g* for 10 min, and one at 2 000 *g* for 30 min.

Serum lactic dehydrogenase (SLDH) and serum hydroxybutyric acid dehydrogenase (SHBDH) activity were measured by the method of Wroblewski and La Due (17) using commercial reagents (Kabi). The upper normal limit of SLDH in our laboratory is 200 U/l. The SLDH activity was measured in all subjects with the mentioned time intervals; in 29 subjects SHBDH activity was measured simultaneously. Serum glutamic oxaloacetic and serum glutamic pyruvic transaminase (SGOT and SGPT) were determined in seven, and creatinine phosphokinase (SCPK) in fourteen cases at the same instants. Measurements of SLDH isoenzymes have not been performed.

The statistical comparisons were made using the *t* test.

RESULTS

Plasma heme changes after exercise

The benzidine method is claimed to have an accuracy better than ±5% with an upper normal value of 5 mg/100 ml (4, 5). In our hands, however, the normal value has been higher and the accuracy lower. Differences between duplicate estimates averaged 0.9 mg/100 ml in the 0–10 mg/100 ml range, and 2.3 mg/100 ml in the 10–20 mg/100 ml range. Our normal mean value was 5.1 mg/100 ml with a standard deviation of 2.7 mg. Therefore, we considered only values above 8.5 mg/100 ml to be pathological.

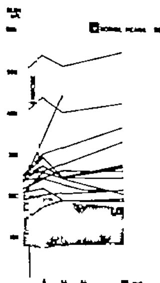


Fig. 1. Changes in SLDH during and after exercise. Unbroken lines – SLDH levels in the individual patients with aortic ball valves. Stippled area – values of controls (mean ±2 S.D.).

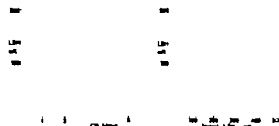


Fig. 2. Left: maximal increase in SLDH after exercise plotted against the increment of cardiac output during exercise. Right: maximal increase in SLDH plotted against the initial resting level of SLDH.

No significant changes of plasma heme concentration were found during or after exercise in any of the groups (Table I). The resting heme level did not differ significantly in groups I and II, and no difference between the different types of aortic valvular disease was found. The mean value of all measurements in group II was slightly but significantly elevated as compared to the other groups. The differences between the groups were too small to be used in the analysis of the individual cases; no really high values were found even in group III (range 2–15 mg/100 ml).

SLDH changes after exercise

The SLDH values at rest did not differ significantly between groups I and II, and were within normal limits (Table II). No significant changes were observed after exercise in either group. In group III, however, the initial values were significantly elevated, in all but two cases exceeding the upper normal limit. All fourteen patients exhibited a smaller or greater increase in SLDH activity during exercise, averaging 33 U/l (Fig. 1). This rise is statistically significant ($P < 0.01$). After the initial rise the curves differed considerably from case to case. Some patients showed a persistent increase throughout the two-hour period, while others reached the initial level after 30 min. No correspondence was found between the numerical increase after exercise and the resting SLDH level (Fig. 2).

Two normal subjects performed an additional submaximal exercise of 1500 kpm/min for 15 min. The increase in SLDH activity was 11 and 27 U/l, respectively, but the fluctuations were well within the normal range.

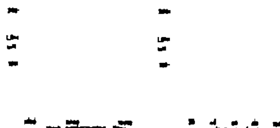


Fig 3 Left: maximal increase in SLDH after exercise plotted against the amount of work performed during the test. Right: maximal increase in SLDH plotted against the increase of heart frequency

No changes in SGOT SGPT or SCPK were found after exercise. All eight patients in group III in whom SHBDH was measured showed an increased activity which paralleled the increment of SLDH, averaging 90% of the SLDH increase. Although isoenzyme studies have not been performed, this indicates that almost all the SLDH increase lies in the anodal 5-fraction.

The increase in SLDH was compared with the hemodynamic parameters measured during exercise (Figs. 2 and 3). No consistent relationship to any of the hemodynamic variables was found. Nor was there any relationship between the LDH increment and the type or size of prosthesis (Fig. 4) The number of patients with each type of valve, however was very small. The patient with a fixed rate pacemaker (rate 67/min) showed an average increase in SLDH.

DISCUSSION

Increased amounts of plasma heme pigments have been reported in several patients with prosthetic aortic ball valves (6, 7 9 14). The reported

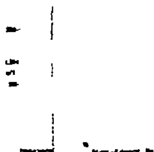


Fig 4 Maximal increase in SLDH after exercise in different valve types and sizes. The size of the Starr-Edwards valves increases with increasing valve number

heme concentrations have ranged from 0 to 250 mg/100 ml, and average values exceeding 30 mg/100 ml have been found for all types of valves (9). The only published series with low values comparable to ours is six patients with Magovern prosthesis in whom the mean plasma heme concentration was 9 6 mg/100 ml (6). Obviously most of the reported patients with grossly elevated values have been studied because severe hemolysis was present, and they are therefore not representative of unselected patients with ball valves.

We, too have seen occasional patients with severe hemolysis and definitely elevated values of plasma heme But in the sixteen patients reported here only a slight elevation was found. Thus it seems that the plasma heme level is not a sensitive indicator of hemolysis.

The different levels of plasma heme found in various studies using similar methods may reflect true differences, but technical errors in the procedure must also be taken into consideration. Almost all these errors tend to give falsely elevated values, the most important being hemolysis during the sampling and the initial handling of the blood (3 4 5) It seems possible that technical errors are responsible for some of the high values reported.

Studies of plasma heme concentration following exercise have previously been made only in a few patients. Sears and Crosby (14) found a significant increase from 50.6 to 64.3 mg/100 ml after several hours of moderate activity in one patient with a Teflon patch in the inter atrial septum. In another patient with an aortic ball valve prosthesis a non-significant increase from 39 to 44 mg/100 ml was found. The difference between their results and ours may be explained by the fact that both of their patients had severe hemolytic anemia and that the period of exercise was longer Ravenel et al. (12) demonstrated an increase in plasma heme during exercise from 4 to 9 mg/100 ml in one unoperated patient with aortic coarctation, with normalization after operation. We have not found such an increase of plasma heme in patients with unoperated, severe aortic valvular disease.

The lack of significant hemoglobinemia even in cases with definite hemolysis is not clearly understood. Probably the most important factor is altered plasma hemoglobin turnover in pa-

tients lacking haptoglobin (4). This factor deserves further investigation.

The most conspicuous finding in this study was the previously undescribed augmentation of SLDH in patients with ball valves when exposed to physical exercise. In contrast, SLDH in the controls as well as in the unoperated patients with aortic valvular disease remained unchanged under similar circumstances. Therefore it seems probable that the ball valve protheses are in some way responsible for the increased enzyme activity after exercise.

The influence of exercise as such on serum enzymes in normal subjects has been investigated by Nerdrum and Berg (11). They found that a brief maximal exercise provoked a slight fall in SLDH, while strenuous exercise for one and a half hour caused a significant rise in SLDH averaging 35 U/l immediately after the test period. In our study the exercise test was far from maximal in most subjects, and the design of the test was more similar to the short-time test used by Nerdrum and Berg. Therefore we do not think that exercise as such was an important cause of the SLDH increment in our patients, although it may have been a contributing factor in some of them.

The proportionate increase in SHBDH points towards the 5-fraction (modal) as the most important contributor to the rise in total SLDH in our patients. This fraction is mainly found in the myocardium kidneys and erythrocytes. None of the patients exhibited signs of either myocardial or renal damage during or after the test, which is consistent with the view that damaged red blood cells is the source of the enzyme.

Other organs which mainly contain other LDH fractions also seem improbable as the origin of the enzyme. The lack of liver congestion or rise in serum transaminases makes hepatic origin unlikely. No considerable trauma to the muscles took place during the exercise, and SCPK did not increase.

Elevation of serum enzymes does not, however necessarily imply organ damage. Metabolic changes during exercise as reflected in hypoxia, acidosis and catecholamine release have been proposed as causes of serum-enzyme elevations after exercise (11). Activation, reduced inactivation and altered enzyme metabolism must also be taken into account. It seems highly improbable, however that such factors operate only in cases with ball valves. Therefore we maintain that mechanical destruction of erythrocytes by the ball valve is the most probable cause of the increase.

Why does a moderate extra hemolysis cause a significant rise in SLDH but no rise in plasma heme pigments? The discrepancy may partially be explained by altered heme catabolism in ahaptoglobinemic patients. Another possible factor may be that LDH is more easily liberated from the disrupted red cells than hemoglobin. Thus SLDH seems to give a more correct picture of the degree of hemolysis during exercise than plasma heme.

After the initial rise, the course of the SLDH curves differed from patient to patient. In some patients a rapid fall in activity was found within 30 min after the exercise. As the serum half life of the SLDH 5 fraction seems to be about 65 hours (15) the fall is probably not due to elimination, but to dilution in extracellular body fluid. A continuing increase in SLDH, as seen in some patients, might be interpreted as liberation of LDH from partially damaged red cells which were able to keep their enzyme content for a short time.

The LDH content of erythrocytes is about 100–150 times that of plasma (17), i.e. about 15 000 U/l. Extra destruction of 10 ml erythrocytes from 20 ml blood during the exercise would then give an increase in SLDH of about 50 U/l, if the enzyme is primarily distributed in plasma. As some of the liberated enzymes may have been distributed to the extracellular body fluid during the exercise period before the first blood sample is taken, this may correspond to the average measured increase in SLDH of 33 U/l.

Several theories have been held concerning the actual mechanisms involved in hemolysis caused by ball valve protheses. Recent in vitro experiments point to the direct contact between the red cell and the surface of the inserted device as the most important factor (1). W hoped that correlation f the calculated amount f destroyed red cells during exercise to various hemodynamic factors would throw further light on the most important mechanical factors. However neither hemodynamics nor ball valve size seemed to influence the degree of hemolysis systematically. This means that unknown individual factors re mainly responsible for the degree of hemolysis

Two such factors are known paravalvular leakage and ball variance, which both may cause severe hemolysis. In this study however no signs of such complications were found. This emphasis on unknown factors is further supported by our observation of severe life-threatening hemolysis in one patient, not included in this study. Subsequent autopsy showed no mechanical defect of the prosthesis. Similar conclusions may be drawn from observations made in other centers showing that reoperation with change of valve often does not remove the hemolysis (13).

Although no correlation between the degree of hemolysis and hemodynamic factors was found between patients, it still seems probable that such correlation exists in the individual patient. This deserves further clarification through a study with similar design of test to that used by us. Restrictions of physical exercise should therefore still be a part of the conservative treatment of patients with hemolytic anemia caused by prosthetic ball valves (13).

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THE USE OF AN ELECTRODE CATHETER IN THE DIAGNOSIS OF EBSTEIN'S ANOMALY OF THE HEART

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Abstract. A case of Ebstein's anomaly of the heart in 53-year-old man is presented. Except for exertional dyspnea, the patient did not present symptoms or signs of manifest heart failure. The diagnosis was strongly suspected through the demonstration of change in pressure patterns from ventricular to atrial when an electrode catheter was withdrawn from the right ventricle across the tricuspidal valve area into the right atrium, although the simultaneously recorded intracardiac ECG remained ventricular in form. The diagnosis was confirmed by the angiographic picture showing moderately enlarged right atrium with displacement of the tricuspidal valve into the ventricle.

The rare congenital malformation of the heart originally described by Ebstein in 1866 (1) is characterized by a displacement of the apposition of one or more of the tricuspidal leaflets to the myocardial wall. The right ventricle in this way is divided into two chambers. The distal chamber is in the region of the right ventricular outflow tract, with walls of normal thickness, and a proximal chamber with thin walls is continuous with a dilated and generally hypertrophied right atrium.

CASE REPORT

In 53-year-old man heart murmur of unknown etiology was heard for the first time when he was 20 years old. He was not restricted in physical activities before 1966, when during four weeks period he developed exertional dyspnea and fatigue and was hospitalized. A blowing systolic murmur was at that time heard to the left of the sternum together with short diastolic murmur. The ECG showed atrial fibrillation. The heart volume was 650 ml/m² on X-ray and an enlargement of the left atrium and right ventricle was suggested. Cardiac catheterization after conversion to sinus rhythm indicated elevated pulmonary artery pressure, with normal cardiac output (resting conditions). After this examination he was practically asymptomatic and able to live fairly active life. A second evaluation of the heart function was made in September 1967.

The physical examination showed normally developed man without cyanosis or edema. The blood pressure was normal. The pulse was regular 76 per min. The peripheral pulses were palpable. There was pansystolic murmur grade II, between the apex and the left sternal border accompanied by an early diastolic murmur. The first and second sounds were normal (Fig. 1). A 12-lead ECG showed sinus rhythm, incomplete right bundle branch block, and low voltage in the medial precordial leads.

Chest X-ray showed normal lungs and pulmonary vascularity. The heart silhouette was normal without any characteristic configuration, and the volume as 520 ml/m² BSA (Fig. 2).

The results obtained by an ordinary right-sided cardiac catheterization t rest and during exercise are given in Table I. Simultaneous recordings of intracardiac pressures and intracavitary potentials were made when the catheter with an electrode on the tip was withdrawn from the right ventricle across the tricuspidal valve area into the right atrium, and are shown in Fig. 3. Repeated

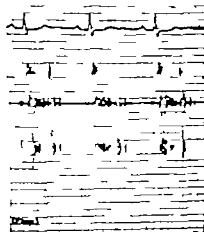


Fig. 1. Phonocardiogram from apex showing holosystolic murmur and presystolic (diastolic) murmur.



Fig 2 a. Posteroanterior X-ray of the chest.

ions demonstrated change in pressure patterns om ventricular to atrial, although the simultaneous intracardiac ECG remained entricular in form. The catheterization thus demonstrated proximal ventricular chamber with ventricular potentials but atrial pressures.

Angiocardiography was performed with right atrial injection and demonstrated an enlarged atrium. The tricuspid valv was displaced to the left of where the right atrioventricular junction is normally located.

DISCUSSION

Clinically the patient described did not present symptoms or signs of manifest heart failure, except for some exertional dyspnea. The cardiac catheterization showed essentially normal haemodynamics, except for a subnormal cardiac output at rest. During an exercise test of moderate intensity the increase in cardiac output was normal, while the pressure in the right atrium was slightly elevated. The pulmonary capillary pressures rose initially to an abnormal level, but fell again at the end of the exercise period. The diagnosis of Ebstein's anomaly was strongly suspected on the basis of the results with the use of an electrode catheter to record potentials and pressures simultaneously. When the catheter was slowly drawn from the right ventricle into the atrium, a distinct change in pressures from ventricular to atrial in an area just below the right ventricular outflow tract was demonstrable, while the intracardiac potentials remained of ventricular form. The point where the pressures suddenly change is most likely the area where the displaced tricuspidal valve divides the right ventricle into two chambers. The diagnosis was confirmed by the angiocardiographic picture, showing the displacement of the valves downward and to the left into the right ventricle.

For several years the rare anomaly of Ebstein (1) was only diagnosed pathologically. The first case diagnosed during life was published in 1949 (10), and later reports have brought the total number up to about 300 cases (3, 7, 8, 11). In the typical cases the characteristic findings that have led to the correct diagnosis have been the coexistence of a systolic murmur heard in the apex region or to the left of the sternum, and more unusually a diastolic murmur in addition, right bundle branch block and low voltage in

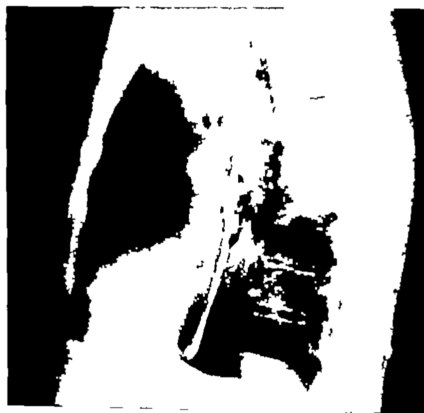


Fig. 2 b. Lateral X-ray of the chest.

the medial precordial leads in the ECG and a globular appearance of the heart, with a normal to decreased vascularity of the lungs on the chest X-ray (5). The diagnosis is substantiated by the angiocardiographic picture, showing an enlarged right atrium with displacement of the tricuspid valve.

While the clinical diagnosis in the typical cases of Ebstein's anomaly can be made with a high degree of accuracy considerable difficulties exist in patients where the malformation is less pronounced, and where the clinical picture may be confused with other heart conditions. It was there-

Table I. *Catheterization data*

	Rest	Exercise (150 kg-m/min in 5 min)
Blood gases, HbO_2 %		
Femoral artery	95.0	94.8
Pulmonary artery	62.5	43.0
Right atrium	63.7	
Superior cava	55.0	
Pressures, Hg mm		
Right atrium	5	10½
Right ventricle	25/4	
Pulmonary artery	27½/10, 17½	41½/17
Pulmonary capillary (PCP)	8	18, 17 14
CO/CI	4.3/2.2	7.7/3.9

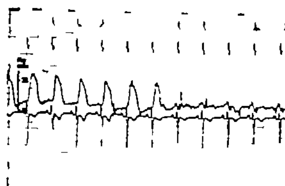


Fig. 3. The tracings illustrate the precordial ECGs (top), pressure recordings (middle) and intracardiac ECGs (bottom) when the electrode catheter is withdrawn from the right ventricle (left) into the right atrium (right). The intracardiac potentials continue to show right ventricular patterns, despite the pressure change from ventricular to atrial.



Fig. 4 Right anterior oblique angiocardiogram following injection of opaque medium into the right atrium. The right atrium is enlarged, and the tricuspidal valve is displaced downwards to the left into the right ventricle.

an advance when Sodi-Pollares and Marsico (9) published their experience with the electrode catheter as an aid in the diagnosis of Ebstein's anomaly The usefullness of their technique has later been demonstrated in examinations of typical cases (6, 12). As shown in the present report, this method may lead to the correct diagnosis even in cases where the clinical picture is less distinctive.

It is obvious from the literature cited that wide variations in functional impairment exist in cases with Ebstein's anomaly The severity of the heart failure depends upon the degree to which the abnormal valve is displaced into the right ventricle. Further on, the condition of the valve, and the size of the distal functioning portion of the ventricle together with the condition of its musculature, are also of importance (2, 4). The present case demonstrates a combination of a tricuspidal valve stenosis and insufficiency with a moderately enlarged right atrium but with a nearly adequate right heart function both at rest and during exercise.

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PNEUMOCOCCAL SEPSIS WITH GENERALISED SHWARTZMAN REACTION

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Abstract. A patient in hospital developed acute pneumococcal sepsis during which lesions typical of the generalised Shwartzman reaction appeared. The patient went into shock, showed signs of respiratory distress, she had haemorrhagic necrosis of the skin and transient anuria but ultimately recovered. Detailed investigation of the haemostatic and fibrinolytic systems revealed rapid consumption of platelets, decreased prothrombin and factor V and large increase of fibrinolytic split products.

In many acute inflammatory and traumatic conditions platelets are aggregated, and the haemostatic and fibrinolytic mechanisms are activated. This leads to a consumption syndrome with a decrease of the number of platelets, of fibrinogen and of other coagulation factors (1 2, 7 10, 34 35). The clinical picture varies, in some patients excessive activation of the fibrinolytic system and the interference with the clotting system and thrombocytopenia may produce severe bleeding (36, 37), while in others widespread microthrombi may produce lesions characteristic of the Shwartzman reaction (15 19 22, [illegible]3 33 34). The severity and acuteness of these conditions require immediate treatment, which, since special laboratory facilities are often not available, is often only based on clinical judgement. It is therefore not surprising that the treatment given varies from one centre to another. There is accordingly need for investigation of such patients with all modern laboratory methods available for the assay of the haemostatic function.

This paper reports a case in which acute pneumococcal sepsis with signs and symptoms typical of a Shwartzman reaction developed in a patient who had been admitted to hospital because of a sore mouth, probably due to folic acid deficiency but who was otherwise in good general condition. Valuable laboratory information had been collected before the onset of the disease and the haemostatic and fibrinolytic systems were studied in detail during the acute stage and convalescence.

METHODS

Platelets were counted by the method of [illegible] (5). Determinations of the bleeding time according to Duke, the coagulation time in glass and plastic tubes, recalcification time, factor VIII, factor IX, one-stage prothrombin time, prothrombin + f. VII + f. X (Owren's P & P test), factor V ere made by the methods described earlier (8, 28, 29). The fibrinogen, euglobulin clot lysis time, fibrinolytic activity of plasma and resuspended euglobulin precipitate on heated and unheated bovine fibrin plates, plasminogen, inhibitors of plasminogen activation and thrombin time were determined as in earlier investigations (30, 31 32). Fibrinolytic split products were identified and quantitated by immunological methods (26, 27).

CASE REPORT

An unmarried, retired nurse, aged 70, who had for several years used barbiturates daily was admitted to hospital in 1961 because of precordial pain, but no signs of myocardial infarction were found. In 1963 she was again admitted to hospital, this time because of soreness of the mouth and suspected anaemia. No signs of B_{12} deficiency could be demonstrated, but the soreness promptly responded to B_{12} therapy. After the patient had been sent home, low level of the folic acid in the serum (0.3 μg l) was recognized, but no specific treatment as given. In Oct. 1967 the patient again complained of glossitis. Treatment ith folic acid was started on Nov 23, but on the following day the patient was nevertheless admitted for observation. She was then in comparatively good health but had papillary atrophy of

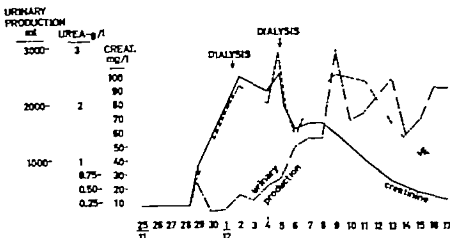


Fig. 3 Urinary production and serum levels of creatinine and urea in the acute disease.

but rose to 72 ml/min 1.73 m² by Feb. 27 The urinary sediment in the acute stage contained numerous red and white blood cells as well as cylinders of hyaline, granular or waxy type. There was slight proteinuria and urinary density of 1.012. With returning renal function the sediment cleared up. Renography on Dec. 4 showed *horizontal curves indicating no uptake of the test substance*, but on Jan. 5 the renogram was normal. During convalescence the density of the urine varied between 1.004–1.020.

Therapy with heparin and dicoumarol was not instituted until Dec. 7 when it was given as prophylaxis against venous thrombosis until the patient was fully mobilised.

In the skin of the limbs and face haemorrhagic necroses developed in the areas that had previously shown the most marked signs of circulatory disturbance. These lesions afterwards healed.

Liver function was only mildly affected, the bilirubin never exceeding 5 mg l. In the acute stage transaminases, SGOT 306, SGPT 169 and SGT 320 increased, but rapidly returned to normal.

ECG showed slight ST depressions in the first few weeks, but the changes were unspecific.

Haematological investigation

The laboratory data obtained before the onset of disease did not suggest any serious abnormality. The low sedimentation rate, 26 mm/hour excluded an abnormal increase of fibrinogen. The platelet count was at the upper normal level (314 000/μl). There were no signs of hepatic or renal disease. It is therefore probable that also haemostasis was normal. The patient had never before been troubled by bleeding or thrombosis.

In the acute stage of the sepsis there were signs of consumptive coagulopathy (Table I) with prolongation of the coagulation time, steep fall in the number of platelets, decrease in the prothrombin group, P & P 37%, and decrease in factor V. The fibrinogen level was 0.33 g/100 ml. The level of fibrinolytic split products was highly increased in the circulating blood. Plasminogen was almost normal. No fibrinolytic activity could be demonstrated in the blood judging from tests on fibrin plates and determination of the euglobulin lysis time.

Thrombocytopenia persisted for six days, after which the number of platelets rose to exceed the normal level (Table I, Fig. 1). The other coagulation factors recovered earlier and also increased to hypernormal values.

The W.B.C. before the onset of the disease was normal, 12 700/μl in the acute stage, after which it increased further to maximum of 27 000/μl, and then gradually returned to normal (Fig. 2).

The haemoglobin was normal (135 g/l) at the time of admission and rose to 152 g/l in the acute stage owing to haemoconcentration, was 124 g/l on Dec. 5 after which it gradually fell to 67 g/l without signs of haemolysis (Fig. 2). It remained at this low level until Feb. 12, when it gradually increased to reach 90 g/l on March 11. The patient then received two pints of blood before being sent home. The haemoglobin level has since remained normal.

DISCUSSION

The generalised Shwartzman reaction was originally produced by two intravenous injections of endotoxin given at an interval of 24 hours. It is characterised by renal cortical necrosis and capillary thrombi with haemorrhagic necrosis of various organs and of the skin. Similar lesions can be produced by exchanging the preparatory or challenging dose of endotoxin by a number of other chemical or biological products *(16, 39)*, by combined injection of thorotrast and endotoxin (38) or a single injection of Liquoid (11). Pre-treatment with heparin or other anticoagulants prevents the syndrome (14 40), and it is generally agreed that intravascular coagulation with fibrin deposits in the smaller vessels constitutes the final common pathway The reactions provoked by endotoxin appear to require the

presence of leucocytes (12, 17 41). Evidence that platelets are of importance has been reported (20). Production of the syndrome by liquoid requires neither leucocytes nor platelets (11). It has been suggested that the reticulo-endothelial system plays an important role in clearing the blood from fibrin deposits and that blocking of phagocytosis by the first injection of endotoxin or thorotrast renders the animals sensitive to the second dose of endotoxin (18).

Though the Shwartzman reaction is essentially an experimental reaction provoked in laboratory animals, equivalent reactions can be seen also in human disease (15 19 23 33 34, 41). The full syndrome is rare, but less pronounced reactions f similar type seem to be common in septic and traumatic conditions, the full syndrome being prevented by inhibitory mechanisms present in the human body In septic conditions thrombo-haemorrhagic phenomena are most commonly encountered in meningococcal sepsis (1 10, 21), but may be provoked also by many other Gram positive and Gram negative bacteria (7).

In this patient the lesions were typical of the Shwartzman reaction with disturbed renal function with *transient anuria*, typical haemorrhagic necrosis of the skin, respiratory distress, mental confusion, and an increased amount of liver enzymes in the circulation. The haemostatic function was compatible with such a reaction and showed a rapid fall in platelets from 315 000 to ‿8 000 per μl. The prothrombin group was decreased so that P&P was only 32% and factor V was 4‿%. All these factors are consumed by coagulation. One might therefore have expected also the fibrinogen to be decreased, but it was not. As there was a sharp increase in the fibrinolytic split products, increased production and mobilisation had evidently balanced the consumption. N fibrinolytic activity was detected on fibrin plates, and the fibrinolytic split products had probably derived from local dissolution of fibrin deposits.

A related condition, thrombotic thrombocytopenic purpura, has haemolysis as one of its main features (24), but there was no indication of haemolysis in our patient.

As the patient survived, no patho-anatomical diagnosis was obtained, and renal puncture was considered too risky The bilateral absence of renal uptake at renography the urinary sediment, and the course with transient anuria suggest that the renal lesions were of Shwartzman type The dextran administered was not of the low molecular type that has rarely been associated with renal damage

The syndrome in this case had evidently been precipitated by the pneumococcal sepsis, as in the case described by Ratnoff and Nebehay (35). It was difficult to recognise any preparatory mechanism, but it should be recollected that treatment of a probably folic acid deficiency had been started three days earlier and that barbiturates, to which the patient was used, had been withdrawn since two days. In view of the rapid reduction of platelets relative to the plasma factors in this case it is tempting to assume that the platelets play a substantial role in the production of lesions, but, as has already been stressed, the basic mechanism is complex and many factors interact.

In animal experiments pre-treatment with heparin or other anticoagulants has proved to block the Shwartzman reaction (14 40). Good results with such treatment have been reported in patients with chronic consumption syndromes, which might occur in malignant disorders (25). In the acute disease heparin treatment has often been recommended (1 7 21 33), and even to patients where bleeding was the dominant symptom (13), but the results have been inconsistent (6), the mortality of treated patients high (4 out of 5 in one of the series) and control material absent or insufficient. The problem is that the action of heparin is mainly prophylactic and if it is to have the desired effect it should be given early in the disease, because late in the disease it may accentuate the then existing bleeding tendency (6). In this case no heparin was given, because when the condition was recognised, the platelet count and prothrombin level were so low that it was feared that heparin might do more harm if bleeding was provoked. It was therefore considered sufficient to give dextran in order to alleviate the shock by increasing the blood volume and reducing the haemoconcentration, but also for its potency to decrease platelet adhesiveness and for its weak anticoagulant properties (7). The dextran given was clinical dextran, Macrodex® (Mw 70 000), the effect of which is quite different from that of the very high molecular dextran (Mw 1 000 000) which is never used clini-

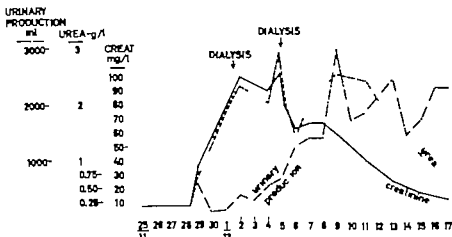


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DERMATITIS HERPETIFORMIS ENTEROPATHY AND WHIPPLE'S DISEASE

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Abstract. A patient with Whipple disease and intestinal villous atrophy for many years developed typical skin lesion of dermatitis herpetiformis. This suggests that the intestinal manifestation might precede the dermal changes of dermatitis herpetiformis. Further the relationship between dermatitis herpetiformis enteropathy and Whipple's disease is discussed.

Intestinal mucosal changes have been demonstrated in a large number of patients with dermatitis herpetiformis without diarrhoea or malabsorption (3). Development of typical dermatitis herpetiformis has been seen in patients who have suffered from idiopathic steatorrhoea (coeliac disease) with villous atrophy for several years despite treatment with gluten-free diet (1, 5).

This is a report on a patient with Whipple's disease who had marked villous atrophy of the jejunal mucosa many years before he developed a typical dermatitis herpetiformis. As far as we know this association has hitherto not been described.

CASE REPORT

The patient is 57-year-old farmer who was admitted to our hospital for the first time in August 1962 because of Whipple's disease. Details were published in 1964 (6).

Since 1949 he had had periodic eruptions of an uncharacteristic eczema in the face, on the hands, legs and perineum.

He complained of intermittent abdominal discomfort and anorexia since 1952. In 1954 he was treated for iron-deficiency anaemia and hypoproteinaemia. In 1961 he had diarrhoea of several months duration. On admission in August 1962 he suffered from fever migratory arthritic pains, weight loss, weakness and diarrhoea.

Physical examination revealed generalized lymphadenopathy marked hyperpigmentation of the skin, neurodermatic changes of hands and face, normochromic anaemia with Hb concentration of 8.5 g/100 ml, ESR 100 mm, hypoalbuminaemia, increased faecal excretion of fat and nitrogen (32 and 3.5 g/24 h, respectively), impaired resorption of vitamin A and xylose and flat glucose tolerance curve.

The diagnosis of Whipple disease was confirmed by the demonstration of PAS-positive material in macrophages of the jejunal mucosa, in the basal layer of the epidermis and in several lymph nodes. The small bowel biopsy with Crosby capsule showed marked villous atrophy of the jejunal mucosa.

There was no clinical effect of gluten-free diet, but following fat restriction there was significant improvement of the diarrhoea. His general condition, however deteriorated until antibiotic treatment was started with penicillin and streptomycin for two weeks, followed by tetracycline (Ledermycin®). After few weeks of this therapy improvement occurred, with normalization of the blood values, weight gain and marked decrease of the faecal fat and nitrogen loss. The lymphadenopathy and the dermatitis persisted unchanged. After treatment for two years tetracycline was discontinued. Relapse occurred after eighteen months and second course of tetracycline therapy was started with good clinical response.

After the first course of tetracycline treatment the PAS-positive material has not been demonstrable in lymph nodes or small bowel biopsies.

During the summer of 1968 he developed skin disease diagnosed at the Department of Dermatology of the University Hospital as typical dermatitis herpetiformis. After treatment with diphenylsulphonodiamine (Dapson) for some months, the dermatitis disappeared. Relapse has not occurred during the first six months after the drug was discontinued.

He is now symptom-free, and there is nearly normal excretion of fat and nitrogen in the stools. The resorption of vitamin A and xylose is, however, moderately decreased. Biopsy from the intestinal mucosa shows persistent total atrophy of the villi. Quantitative determinations of the different immunoglobulins were made by single radial immunodiffusion in agar gel (4). The level of IgM was markedly decreased (26 mg/dl), whereas the concentration of IgA was slightly increased (390 mg/dl). IgG was in the normal range (1 090 mg/dl).

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α-FETOPROTEIN IN HEPATOCELLULAR CARCINOMA WITH AN ABNORMAL ELECTROPHORETIC α_1 FRACTION

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Abstract. A very high serum concentration of α-fetoprotein (156 mg/100 ml) was demonstrated in a 62-year-old man with advanced hepatocellular carcinoma and liver cirrhosis. The protein produced a distinct band in agarose gel electrophoresis which was indistinguishable from that seen in certain cases of hereditary electrophoretic abnormality of α_1-antitrypsin.

Pedersen (15) described the occurrence of an embryonal protein in fetal calf serum. He called it fetuin. Electrophoretically it belonged to the α_1-fraction and had a molecular weight of about 50,000. A fetuin homologue has since been demonstrated in fetal sera of several other mammals (9, 12, 20) including man (4). In recent literature this protein is usually called α-fetoprotein. With sensitive immunochemical methods it can be demonstrated in human fetal plasma already after a few weeks gestation. Its concentration is highest, about 300 mg/100 ml, between the 10th and the 13th week. The fraction is then demonstrable as a distinct band between the albumin and α_1-globulins in the electrophoretic pattern (Fig. 1). It afterwards decreases rapidly in concentration but traces of it may still be demonstrated in the first two weeks after birth (4, 8, 14, 18).

Abelev et al. (2) in 1963 demonstrated the presence of α-fetoprotein in plasma of adult mice with experimentally induced hepatoma, and Tatarinov (17) in 1964 found α-fetoprotein in serum from a patient with primary hepatocellular carcinoma. The method used by these authors for the demonstration of the protein was an immunochemical double diffusion technique essentially according to Ouchterlony. Since then some large clinical series have been presented (1, 3, 7, 11, 13, 16, 19) which have shown that modifications of this method can demonstrate α-fetoprotein in plasma from 30–80% of all patients with hepatocellular carcinoma, and that it does not occur in healthy adults or in adults with other diseases studied, with the exception of patients with teratomas and teratoblastomas of embryonal type. In newborns and in infants below one year α-fetoprotein is occasionally found also in other liver diseases.

With this method it is possible to demonstrate the presence of α-fetoprotein in a concentration of at least 1 mg/100 ml (5). Some investigators determined the concentration of α-fetoprotein in the positive cases and, as a rule, it was found to be below 10 mg/100 ml. However in a 15-year old girl with malignant teratoblastoma of the ovary it was 27 mg/100 ml, and in a 14-year old boy with hepatocellular carcinoma it increased during the last six months of life from 20 to 90 mg/100 ml (10, 13). Foli et al. (7) recently reported that they had found concentrations up to 150 mg/100 ml but no clinical data were presented. In plasma from a 62 year old man with primary hepatocellular carcinoma we have recently observed a very high concentration of α-fetoprotein producing a distinct abnormal band between the albumin and the α_1-globulin bands of an otherwise normal electrophoretic pattern.

CASE REPORT

The patient was a man, born in 1907. He had been a heavy drinker for several years. He was admitted to the Department of Internal Medicine for the first time in July 1968, because of advanced liver cirrhosis, with

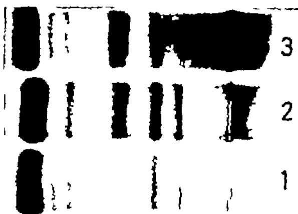


Fig 1 Agarose gel electrophoresis of fetal serum (1), normal adult serum (2) and patient's serum (3).

massive ascites but without hepatosplenomegaly or roentgenographically demonstrable oesophageal varices.

Pertinent laboratory studies during hospitalization. Serum bilirubin 3.0–2.5 mg/100 ml with a positive direct reaction, SGOT 204-71 Karmen units, SGPT 53-32 Karmen units, serum alkaline phosphatase and serum lactate dehydrogenase were within the normal range, serum ammonia 127 µg/100 ml, serum cholesterol 150 mg/100 ml, Owren's prothrombin-proconvertin activity 32%, Hb 9.5 g/100 ml, RBC 2.8 millions mm³ ESR 37-55 mm/h, serum urea 50–53 mg 100 ml, serum electrolytes normal. Repeated tests for blood in the faeces were negative. Serum electrophoresis showed pattern typical of liver cirrhosis with no extra band.

The patient improved on treatment with neomycin, furosemide, spironolactone, digoxine, a sodium- and protein-restricted diet, and multivitamins. After three weeks he was discharged and then followed up at the Out-patient Department. In October 1968 his liver could be felt two fingerbreadths below the right costal arch. Soon afterwards the patient failed to appear for further follow-up.

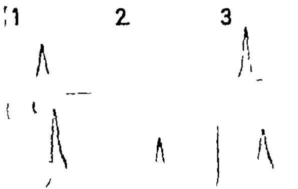


Fig 2. Antigen-antibody crossed electrophoresis* of the sera in Fig. 1 with anti-α-fetoprotein (*above*) and anti-α_1-antitrypsin (*below*).

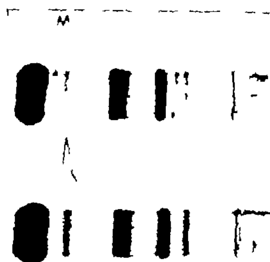


Fig 3 Abnormal α_1-antitrypsin fraction in gel electrophoresis and antigen-antibody crossed electrophoresis (*above*) compared with the patterns of normal serum (*below*).

The patient was re-admitted to hospital in August 1969 He was then emaciated and seriously ill, with cyanosis, dyspnoea, mental disorientation, and extreme weakness. The patient was also icteric and had massive ascites. The liver was felt to be grossly enlarged, nodular and very firm, and to extend below the umbilical plane. Neither the spleen nor any enlarged lymph nodes were palpable. No oesophageal varices were demonstrated.

Laboratory studies. Serum bilirubin 18.5–22.0 mg/100 ml with a positive direct reaction, SGOT 323–219 Karmen units, SGPT 49–168 Karmen units, serum alkaline phosphatase 18–13 units, serum lactate dehydrogenase 2 225 Wróblewski units with elevations of the isoenzyme fractions LDH and, less pronounced, LDH and LDH, serum γ-glutamyl transpeptidase 580 units (normal 15–80), serum amylase normal, serum ammonia 120 µg/100 ml, serum cholesterol 360 mg/100 ml, prothrombin-proconvertin activity 41% Hb 12.0 g/100 ml, RBC 3.6 millions/mm³ WBC 11 700/mm³ with 80% neutrophils, ESR 24 mm/h, serum urea 81 168 mg/100 ml, serum creatinine 2.25–5.95 mg/100 ml, serum potassium 5.7–6.9 mEq/l, sodium 118–134 mEq/l, chloride 95–86 mEq/l, calcium 4.5–4.8 mEq/l, phosphate-P 3.6–9.1 mg/100 ml, standard bicarbonate 25–7 mEq/l, and magnesium 1.9 mEq/l. The tests for blood in the stool were positive.

Serum electrophoresis exhibited a pattern most consistent with severe inflammatory reaction. In addition, between the albumin and the α_1-globulin fractions there was an extra band (Fig. 1). Its concentration was about 160 mg/100 ml. Immunochemical analyses for serum α-fetoprotein gave positive result

The patient gradually deteriorated and died five days after admission to hospital.

Autopsy The liver weighed 4 250 g. It showed signs of cirrhosis, and extremely propagated malignant tumour growth, with infiltration of the portal and hepatic veins.

widespread thromboembolic dissemination in the pulmonary arteries, advanced carcinomatous haemangiosis in both lungs, and metastases in the pulmonary parenchyma and in the hepatic hilar lymph nodes. Microscopic examination revealed marked cirrhotic alteration of the liver architecture, and everywhere in the parenchyma there were numerous foci of hepatocellular carcinoma, with neoplastic liver cells, growing in trabeculae or annular formations, surrounded by wide sinusoid spaces, and heavily infiltrating adjacent blood vessels. The changes in the lungs, pulmonary arteries, portal and hepatic veins, and hepatic hilar lymph nodes were of similar microscopic appearance.

RESULTS AND DISCUSSION

The electrophoretic mobility of the abnormal α_1-band coincided exactly with that of α-fetoprotein in human fetal serum (Fig. 1). The abnormal protein was identified as α-fetoprotein by immunochemical comparison with the fetal serum by antigen-antibody crossed electrophoresis (Fig. 2). The concentration of α-fetoprotein in the patient's plasma was determined colorimetrically after agarose gel electrophoresis, staining with amido black and elution of the abnormal band with an alkaline ethanol solution. Assuming the affinity of amido black for α-fetoprotein to be the same as the mean affinity for all of the plasma proteins the concentration was calculated at 156 mg/100 ml. With one exception (7) no abnormal electrophoretic fraction has been reported in the earlier published cases with a high concentration of α-fetoprotein. This might perhaps be due to the use of electrophoretic techniques with a lower resolution in the albumin-α_1 globulin zones than that of agarose gel electrophoresis. The very high concentration found was probably due to the fact that the patient was observed in a very advanced stage of the disease and that the carcinoma had a strong tendency to grow intravascularly with widespread thrombo-embolic dissemination. The demonstration of abnormal electrophoretic bands in the albumin-α_1-globulin zone is therefore probably of value in the diagnosis of hepatocellular carcinoma only in very advanced cases and the diagnosis can never be established solely on such an electrophoretic finding. For abnormal electrophoretic bands in this zone are occasionally seen in sera from apparently healthy subjects and are due to hereditary abnormalities of α_1-antitrypsin (6) (Fig. 3).

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ORGAN-SPECIFIC, ANTIRENAL CELLULAR HYPERSENSITIVITY AFTER KIDNEY TRANSPLANTATION

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Abstract. The in vitro reactivity of peripheral leucocytes to renal parenchymatous extract has been examined by means of a capillary tube migration technique (leucocyte migration test, LMT) in 34 patients who had received a renal transplant. In eight patients with uneventful course, and in the remaining 26 patients during uncomplicated periods (total of 206 observations), no inhibition of the migration was seen. During episodes with reduction in renal function the migration was inhibited in ten of 15 cases with acute rejection. In two cases of chronic rejection LMT was normal. Inhibition in the LMT was seen during two of seven episodes with leakage of the ureteral anastomosis. One of these patients had received a kidney from an identical twin, so ureteral rejection as the cause of the leakage could be excluded. Inhibition could also be demonstrated in a patient with recurrence of the original disease in the grafted kidney (glomerulonephritis). In six patients with transient ischaemic damage of the grafted kidney no inhibition was seen in the anuric period. The reactivity demonstrated in the present study thus indicates the development of organ-specific, antirenal cellular hypersensitivity possibly caused by disintegration of a proportion of the graft cells with release of kidney-specific antigens. In the present modification the LMT seemingly does not reflect hypersensitivity to transplantation antigens for the following reasons: (i) No inhibition was seen with other tissue extracts (which might equally well contain transplantation antigens); (ii) foetal kidney extract was not able to produce transformation of lymphocytes in suspension culture; (iii) patients with active glomerulonephritis also show this reactivity. The conclusion is that LMT with foetal kidney extract reflects renal damage of different aetiology and is therefore of no practical value in the diagnosis of acute rejection after renal transplantation.

Based upon systems indicated and used by George and Vaughan (7), David (4), and David et al. (5) Bendixen and Søborg (1), and Søborg and Bendixen (14) developed a technique for in vitro demonstration of cellular hypersensitivity in man. With this system it has been shown that antigen-induced inhibition of the in vitro migration of peripheral leucocytes is an expression of microbial cellular hypersensitivity (15, 16) and the method has further proved useful for demonstration of various types of organ-specific, cellular hypersensitivity (1, 2, 6, 9, 10, 1.).

In patients who have received a renal transplant, reduction in renal function may be caused by one or more of the following conditions: ischaemic damage to the grafted kidney, rejection (acute or chronic), surgical complications (preeminently leakage of the ureteral anastomosis), severe pyelonephritis, and recurrence of the original disease in the grafted kidney (glomerulonephritis).

The purpose of the present study is to investigate, by the methods of Bendixen and associates, the occurrence of antirenal cellular hypersensitivity to normal kidney in patients who have received a renal transplant, especially during the above mentioned clinical episodes with impaired renal function.

MATERIAL

The material consists of 34 consecutive patients, who underwent renal transplantation (Tables I and II). The diagnoses of the original kidney disease were established on the base of usual objective and anamnestic criteria as well as on histopathological examination. In 15 of 16 patients with glomerulonephritis the symptoms started three years or more before the transplantation. Before transplantation the patients were treated twice weekly with haemodialysis (Kiil or Gambro dialyser). The duration of dialysis was 10–14 hours, and the mean serum creatinine about 8 mg/100 ml. Twelve patients (nos. 4, 7, 23, 4, 6, 27, 28, 29, 30, 32, 33 and 34) had bilateral

Table I. *Data of the 34 patients*

Pat. no.	Age (y)	Sex	Primary kidney disease	Estimated duration of renal disease (y.)	Duration of dialysis treatment before transplantation (mo.)
1	30	♀	Chronic glomerulonephritis	10	12
2	45		Chronic glomerulonephritis	8	2
3	28	♂	Chronic glomerulonephritis	3	4
4	33	♂	Chronic glomerulonephritis	5	4
5	36	♂	Chronic pyelonephritis	18	4
6	26	♂	Chronic pyelonephritis	13	4
7	51	○	Chronic glomerulonephritis	9	24
8	44	♂	Chronic glomerulonephritis	4	7
9	42		Polycystic disease	15	6
10	33		Chronic pyelonephritis	10	2
11	43	♂	Chronic pyelonephritis	3	10
12	23		Chronic glomerulonephritis	20	60
13	20	*	Chronic glomerulonephritis	16	12
14	40	*	Subacute glomerulonephritis	1/2	4
15	45	*	Chronic pyelonephritis	17	36
16	29	♂	Chronic glomerulonephritis	3	2
17	59	♂	Chronic glomerulonephritis	5	12
18	17		Dilaceratio renis traum.	1/4	3
19	51		Chronic pyelonephritis	6	36
20	21	♂	Chronic glomerulonephritis	4	3
21	4		Chronic glomerulonephritis	6	9
22	33	♂	Chronic pyelonephritis	10	40
23	57	♂	Chronic pyelonephritis	10	30
24	40		Chronic pyelonephritis	8	24
25	42		Chronic pyelonephritis	31	5
	46		Chronic pyelonephritis	9	6
	51		Chronic pyelonephritis	7	1
	48	♂	Chronic pyelonephritis	5	12
29	29	♂	Chronic glomerulonephritis	3	1
30	26		Chronic pyelonephritis	15	6
31	44		Chronic pyelonephritis	14	6
32	47		Polycystic disease	33	12
33	53		Chronic glomerulonephritis	30	1
34	52	♂	Chronic glomerulonephritis	3	1

nephrectomy and transplantation performed as a one-stage operation, whereas 22 were nephrectomized before transplantation. Twenty-four patients were treated with extracorporeal irradiation of the blood before the transplantation. The irradiation was performed with 60Co source, a mean duration of the treatment of 100 hours, and mean cumulative erythrocyte dose of 50 000 rads. *Hereby a reduction of the blood lymphocyte count to* about 20% of the pre-treatment values was obtained (19). Ten patients did not receive this treatment (nos. 1, 15 18, 23 4, 26, 28, 30, 31 and 32).

The transplantations were performed with conventional technique, the donor kidney being placed in the contralateral iliac fossa. In 31 patients the pelvis of the donor kidney was anastomosed with the ureter of the recipient. In three patients (nos. 6 and 7) neo-implantation of the ureter in the bladder was performed.

Table II shows the results of the histocompatibility testing of donors and recipients (8) and indicates the condition of the patients 1/2 22 months after transplantation.

The immunosuppressive treatment after the transplantation consisted of azathioprine (Imurel®) and prednisone: the mean dose through the first 12 months is illustrated in Table III (case 1 did not receive immunosuppressive therapy). Twenty-four patients were treated with extracorporeal irradiation for brief periods after the transplantation; among these were three patients (nos. 15 18 and 31) who had not received this treatment before the transplantation. One patient (no. 12) received local irradiation on the graft (150 rads) immediately after the operation.

The diagnosis of graft rejection was made on the basis of conventional criteria, as indicated, e.g., in the review by Starzl (13). In case of acute renal insufficiency in the immediate post-operative course, the patients were always re-operated. If surgical complications could be excluded, renal biopsy was performed.

Table II. *Histocompatibility ranking. Clinical status and immunosuppressive treatment of the 34 patients at the end of the observation period (December 1 1969)*

Pat. no.	Donor	Match[a]	Period of observation (mo.)	Serum creatinine (mg/100 ml)	Creatinine clearance (ml/min)	Blood pressure (mm Hg)	Proteinuria (g/day)	Treatment: Prednisone (mg/day)	Treatment: Azathioprine (mg/day)
1	Related	Identical twin	22	0.9	80	110/70	0	0	0
2	Related	D	18.5	1.3	55	130/80	0	0	100
3	Related	C	15	1 7	60	125/90	0	10	75
4	Related	A_1	14	2.1	69	120/90	0	0	75
5	Related	C	13	1.8	50	110/70	0.4	20	75
6	Related	C	12.5	1.3	83	130/90	0	10	100
7	Necro	D or E	11.5	3.1	40	180/100	0	15	50
8[b]	Related	A	4	1.0	60	120/80	0	15	150
9	Related	C	10	1.8	40	150/100	0	30	100
10	Related	C	9.5	1.6	50	140/95	0.6	12.5	100
11	Related	C	8.5	1.6	60	140/95	0.4	15	125
12	Related	D	7.5	1.0	90	120/80	0	20	75
13	Related	D	7.5	1.2	60	110/70	0	15	100
14	Related	A_1	7	2.9	20	150/100	0.8	30	125
15	Necro	C	6.5	1.9	30	110/70	0	20	100
16	Related	A_1	6	1.3	100	130/90	0	0	125
17	Necro	B	6	2 4	45	160/100	4.2	40	100
18	Necro	B	6	1.0	75	140/90	0	15	100
19	Necro	B or C	6	1 4	55	210/110	0	20	125
20	Related	A_1	5.5	1 1	60	140/100	2.4	60	100
21	Related	A_1	4 5	1 1	70	120/80	0	0	125
22	Necro	D or E	3	1 4	90	160/100	0.7	15	100
23	Necro	C	3	1 7	90	170/80	0	30	125
24	Necro	C or D	2.5	2.9	15	160/100	8.2	250	150
25	Necro	C or D	2 5	1 1	65	170/100	0	30	125
26	Necro	C	2.5	1.6	90	200/120	0	30	75
27	Related	A_1	2	1.2	50	120/80	0.8	15	150
28	Necro	C	2	1.2	65	160/100	0	30	125
29	Related	C	1.5	1 4	95	170/100	0	25	100
30	Necro	B or C	1.5	0.7	95	120/90	0	30	100
31	Necro	D or E	1.5	1.2	45	120/90	0	30	100
32	Necro	C or D	1	2 8	16	160/100	1.3	80	150
33	Related	A_1	1	1 1	70	130/95	3.1	25	150
34	Related	A_1	0 5	1 7	57	125/80	0	30	150

[a] A-match: no incompatibilities between donor and recipient.
B-match: one minor incompatibility between donor and recipient.
C-match: one major incompatibility between donor and recipient.
D-match: two major incompatibilities between donor and recipient.

[b] This patient died of severe serum hepatitis four months after transplantation. The graft functioned well.

METHODS

Leucocyt Migration Test (LMT)

The capacity of components of renal tissue to induce specific inhibition of the migration of peripheral leucocytes was estimated in vitro by means of the LMT which has been described in detail elsewhere (1) and will only be briefly outlined. Heparinized blood from cubital vein is left to form sediment for one hour at 37°C. The plasma is withdrawn and the white blood cells are washed three times in Hank balanced salt solution. The cell suspension is transferred to capillary tubes and the leucocytes (approximately 50% granulocytes and 50% lymphocytes) are allowed to migrate for 24 hours from the open end of the capillary tube along the plain bottom of 1 ml tissue-culture chamber containing TC 199 with 10% horse serum. The circular migration area is measured by paper planimetry. The migration area of antigen-containing cultures (in casu kidney) is compared to the migration area of control cultures without antigen and with other antigens. The two areas (area plus antigen divided by area without antigen or with other antigen) are used to calculate the migration index (MI), which indicates inhibition if the value is below and stimulation if the value is above unity.

Preparation of antigens

Foetal kidneys were recovered and prepared aseptically in order to avoid contamination with bacterial antigens. Kidneys from six foetuses were cut in small bits, pooled,

Table I. *Data of the 34 patients*

Pat. no.	Age (y.)	Sex	Primary kidney disease	Estimated duration of renal disease (y.)	Duration of dialysis treatment before transplantation (mo.)
1	30	♀	Chronic glomerulonephritis	10	12
2	45	♀	Chronic glomerulonephritis	8	2
3	28	♂	Chronic glomerulonephritis	3	24
4	33	♂	Chronic glomerulonephritis	5	4
5	36	♂	Chronic pyelonephritis	18	4
6	26	♂	Chronic pyelonephritis	13	4
7	51	♂	Chronic glomerulonephritis	9	34
8	44	♂	Chronic glomerulonephritis	24	7
9	42	♂	Polycystic disease	15	6
10	33	♀	Chronic pyelonephritis	10	2
11	43	♂	Chronic pyelonephritis	3	10
12	23	♀	Chronic glomerulonephritis	20	60
13	20	♀	Chronic glomerulonephritis	16	12
14	40	♀	Subacute glomerulonephritis	1/2	4
15	43	♀	Chronic pyelonephritis	17	36
16	29	♂	Chronic glomerulonephritis	3	2
17	39	♂	Chronic glomerulonephritis	5	12
18	17	♀	Dilaceratio ren. traum.	1/4	3
19	51	♂	Chronic pyelonephritis	6	36
20	21	♂	Chronic glomerulonephritis	4	3
21	24	♀	Chronic glomerulonephritis	6	9
22	33	♂	Chronic pyelonephritis	10	40
23	57	♂	Chronic pyelonephritis	10	30
24	40	♀	Chronic pyelonephritis	8	24
25	42	♀	Chronic pyelonephritis	31	3
	46	♀	Chronic pyelonephritis	9	6
	31		Chronic pyelonephritis	7	1
	48	♂	Chronic pyelonephritis	5	12
	29	♂	Chronic glomerulonephritis	3	1
30	26	♀	Chronic pyelonephritis	15	6
31	44		Chronic pyelonephritis	14	6
32	47		Polycystic disease	13	12
33	33	♂	Chronic glomerulonephritis	30	1
34	52	♂	Chronic glomerulonephritis	3	1

nephrectomy and transplantation performed as one-stage operation, whereas 22 were nephrectomized before transplantation. Twenty-four patients were treated with extracorporeal irradiation of the blood before the transplantation. The irradiation was performed with ^{60}Co source, mean duration of the treatment of 100 hours, and mean cumulative erythrocyte dose of 50 000 rads. Hereby reduction of the blood lymphocyte count to about 20% of the pre-treatment values was obtained (19). Ten patients did not receive this treatment (nos. 1, 15 18, 23, 24, 26, 28, 30 31 and 32).

The transplantations were performed with conventional technique, the donor kidney being placed in the contralateral iliac fossa. In 31 patients the pelvis of the donor kidney was anastomosed with the ureter of the recipient. In three patients (nos. 5, 6 and 7) a neo-implantation of the ureter in the bladder was performed.

Table II shows the results of the histocompatibility testing of donors and recipients (8) and indicates the condition of the patients 1/2–22 months after transplantation.

The immunosuppressive treatment after the transplantation consisted of azathioprine (Imurel®) and prednisone; the mean dose through the first 12 months is illustrated in Table III (case 1 did not receive immunosuppressive therapy). Twenty-four patients are treated with extracorporeal irradiation for brief periods after the transplantation; among these were three patients (nos. 15 18 and 31) who had not received this treatment before the transplantation. One patient (no. 12) received local irradiation on the graft (150 rads) immediately after the operation.

The diagnosis of graft rejection was made on the basis of conventional criteria, as indicated, e.g., in the review by Starzl (13). In case of acute renal insufficiency in the immediate post-operative course, the patients were always re-operated. If surgical complications could be excluded, renal biopsy was performed.

Table II. *Histocompatibility ranking. Clinical status and immunosuppressive treatment of the 34 patients at the end of the observation period (December 1 1969)*

Pat. no.	Donor	Match[a]	Period of observation (mo.)	Serum creatinine (mg/100 ml)	Creatinine clearance (ml/min)	Blood pressure (mm Hg)	Proteinuria (g/day)	Treatment Prednisone (mg/day)	Treatment Azathioprine (mg/day)
1	Related	Identical twin	22	0.9	80	110/70	0	0	0
2	Related	D	18.5	1.3	55	130/80	0	0	100
3	Related	C	15	1.7	60	125/90	0	10	75
4	Related	A_2	14	2.1	69	120/90	0	0	75
5	Related	C	13	1.8	50	110/70	0.4	20	75
6	Related	C	12.5	1.3	83	130/90	0	10	100
7	Necro	D or E	11.5	3.1	40	180/100	0	15	50
8[b]	Related	A_2	4	1.0	60	120/80	0	15	150
9	Related	C	10	1.8	40	150/100	0	30	100
10	Related	C	9.5	1.6	50	140/95	0.6	12.5	100
11	Related	C	8.5	1.6	60	140/95	0.4	15	125
12	Related	D	7.5	1.0	50	120/80	0	20	75
13	Related	D	7.5	1.2	60	110/70	0	15	100
14	Related	A_2	7	2.9	20	150/100	0.8	30	125
15	Necro	C	6.5	1.9	30	110/70	0	20	100
16	Related	A_1	6	1.3	100	130/90	0	0	125
17	Necro	B	6	2.4	45	160/100	4.2	40	100
18	Necro	B	6	1.0	75	140/90	0	15	100
19	Necro	B or C	6	1.4	55	210/110	0	20	125
20	Related	A_1	5.5	1.1	60	140/100	2.4	60	100
21	Related	A_1	4.5	1.1	70	120/80	0	0	125
22	Necro	D or E	3	1.4	50	160/100	0.7	15	100
23	Necro	C	3	1.7	50	170/80	0	30	125
24	Necro	C or D	2.5	2.9	15	160/100	8.2	250	150
25	Necro	C or D	2.5	1.1	65	170/100	0	30	125
26	Necro	C	2.5	1.6	50	200/120	0	30	75
27	Related	A_1	2	1.2	50	120/80	0.8	15	150
28	Necro	C	2	1.2	65	160/100	0	30	125
29	Related	C	1.5	1.4	95	170/100	0	25	100
30	Necro	B or C	1.5	0.7	95	120/90	0	30	100
31	Necro	D or E	1.5	1.2	45	120/90	0	30	100
32	Necro	C or D	1	2.8	16	160/100	1.3	80	150
33	Related	A_1	1	1.1	70	130/95	3.1	25	150
34	Related	A_1	0.5	1.7	57	125/80	0	30	150

A-match: no incompatibilities between donor and recipient.
B-match: one minor incompatibility between donor and recipient.
C-match: one major incompatibility between donor and recipient.
D-match: two major incompatibilities between donor and recipient.
This patient died of severe serum hepatitis four months after transplantation. The graft functioned well.

METHODS

Leucocyte Migration Test (LMT)

The capacity of components of renal tissue to induce specific inhibition of the migration of peripheral leucocytes was estimated in vitro by means of the LMT, which has been described in detail elsewhere (1) and will only be briefly outlined. Heparinized blood from cubital vein is left to form sediment for one hour at 37°C. The plasma is withdrawn and the white blood cells are washed three times in Hank' balanced salt solution. The cell suspension is transferred to capillary tubes and the leucocytes (approximately 50% granulocytes and 50% lymphocytes) are allowed to migrate for 4 hours from the open end of the capillary tube along the plate bottom of 1 ml tissue-culture chamber containing TC 199 with 10% horse serum. The circular migration area is measured by paper planimetry. The migration area of antigen-containing cultures (in case kidney) is compared to the migration area of control cultures without antigen and with other antigens. The two areas (area plus antigen divided by area without antigen or with other antigen) are used to calculate the migration index (MI), such indicates inhibition if the value is below and stimulation if the value is above unity.

Preparation of antigens

Foetal kidneys are recovered and prepared [illegible] in order to avoid contamination with [illegible] Kidneys from six foetuses were cut in small [illegible]

Table III. *The dosages of prednisone (mg/day) and azathioprine (Imurel*[1]*) (mg/day) employed after renal transplantation. (Mean values ± S D)*

Month after transplantation	1	2	3	4	5	6	7	8	9	10	11	12
No. of pats.	33	27	22	20	18	17	12	9	8	7	6	5
Prednisone (mg/day)	46	29	26	21	19	25	20	16	17	16	10	10
(Mean and S D)	±43	±17	±20	±14	±10	±22	±12	±10	±15	±10	± 7	±20
Azathioprine (mg/day)	118	108	105	103	103	103	98	94	97	89	92	85
(Mean and S.D.)	±31	±27	±20	±17	±17	±17	±20	±21	±21	±24	±25	±14

washed twice in Hank's balanced salt solution, homogenized in a Warren blender at 15 000 r.p.m. for four minutes, left at 4°C for 24 hours, and centrifuged at 1 000 g for 20 min. The supernatant was separated, lyophilized, and stored at 4°C. Before use the lyophilized extract was reliquified with the proper volume of sterile water and the solution was standardized by protein determination (Lowry's method). The concentration of kidney protein in the cultures was normally 100 µg per ml culture medium, which appeared to be the highest concentration not causing non-specific inhibition in normal controls. Extract of adrenal glands was prepared in the same way The highest, non-toxic concentration as measured by protein contents proved to be 200 µg per ml with adrenal extract.

Controls. On the basis of 20 controls the normal range (mean ± 2 S D.) of the LMT with kidney extract was 0.81 to 1.11 (mean 0.96). With adrenal extract normal range of 0.83 to 1.07 (mean 0.95) was calculated on the basis of 18 controls.

The LMT was performed weekly during treatment in the ward, the first examination being made on the third day after transplantation. After discharge the LMT was performed every two weeks as part of the current control in the open ward. Further LMT was performed in relation to all clinical episodes with reduced renal function.

Table IV *Leucocyte migration test (LMT) with extract of foetal kidney in eight patients with uneventful course and in 26 patients during uncomplicated periods after the transplantation*

(Mean values ± S.D.)

Pat. no.	LMT level (Mean ± S.D.)	No. of observations
1	0.95 ± 0.10	12
2	0.95 ± 0.10	24
3	1.03 ± 0.09	17
	1.01 ± 0.11	13
5	1.01 ± 0.10	13
6	1.01 ± 0.12	18
7	0 92 ± 0.06	4
8	0.98 ± 0.06	5
9	1.02 ± 0.09	10
10	0.99 ± 0.07	5
11	0.90 ± 0.11	5
12	1.02 ± 0.09	8
13	0.92 ± 0 07	5
14	1.02 ± 0.13	4
15	1.00 ± 0.10	4
16	1.01 ± 0 09	5
17	1 02 ± 0.15	4
18	1.00 ± 0.04	4
19	0.98 ± 0 09	7
20	1.00 ± 0.11	3
21	1 15 ± 0.20	4
22	1.05	1
23	1.13 ± 0.30	5
24	0.95 ± 0.00	2
25	0.92 ± 0.10	5
26	1 16	1
27	1.01 ± 0.20	6
28	1.11	1
29	0.99 ± 0.00	2
30	0.99 ± 0.10	2
31	0.93 ± 0.11	4
32	0.89	1
33	0.91 ± 0 00	2
34	0.91 ± 0.10	2
Total	0.99 ± 0.11	206

RESULTS

LMT during uneventful course and in uncomplicated periods

The results in eight patients with uneventful course after the transplantation, and in the remaining 26 patients during uncomplicated periods (total of 206 observations), are shown in Table IV The mean value and standard deviation (mean ± S.D = 0 99 ± 0 11) are not significantly different from the normal.

LMT during rejection

1 *Acute rejection.* The findings in 12 patients during 15 acute rejection episodes 3–147 days after transplantation appear from Table V With reference to the usual level of LMT in each patient the LMT was significantly reduced during

Table V *Leucocyte migration test (LMT) with extract of foetal kidney in 12 patients during 15 acute rejection episodes and in two patients with chronic rejection*

Pat no.	Day(s) of rejection episode	LMT
2	20	0.66
2	124–130	0 61 0.74 0 84
3	7–23	0.78
4	51 55	0.79
5	47–55	1 12, 0 87
7	147 160	0 83 0.78
14	7–17	0.86, 0.71 0.81
14[a]	86–	1 02, 1.00
17[a]	64–	0 96, 0 93, 1.09
22	3–5	1.04 1.07
22	87–91	0.61
23	4–7	1 05, 1.15
23	83	0.70
31	6–21	0 82, 1 14, 1.12
32[b]	39–	0.52, 0.48
33	17–21	0.84, 0.87
34	5–12	0.80, 0 89 0.77

Chronic rejection.
Irreversible rejection.

ten of these episodes. The mean dose of glucocorticoid administered at the time of examination was smaller (mean 71 mg prednisone) in rejection episodes which were associated with inhibition in the LMT than in those without inhibition (mean 165 mg prednisone) The mean LMT in 29 examinations during the 15 episodes of rejection was 0.83 (S.D $\pm 0 18$), which was significantly lower than the normal values. Fig. 1 shows the course of LMT and some of the clinical parameters during an acute rejection episode in cases 2 and 14

– Chronic rejection. Four patients have chronic rejection (nos. 7 9 14 and 17). Five LMT examinations in two of these (nos. 14 and 17) were normal (Table V).

LMT d ring other complications

1 *Leakage of ureteral anastomosis* During seven episodes in six patients who developed more or less protracted reduction of the renal function due to leaking anastomosis the LMT was inhibited in two (nos. 1 and 21) out of five examinations

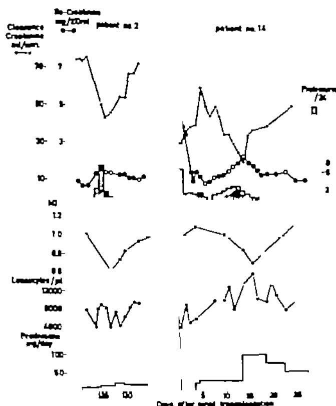


Fig 1 The post-operative course in patients and 14, both of whom had an acute rejection episode 124 and 7 days after the transplantation.

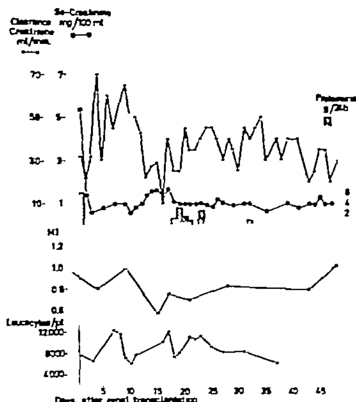


Fig. 2. The post-operative course in patient 1. An ureteral complication 12 days after transplantation caused periodic reduction in renal function.

(Table VI). The course of the LMT in association ith the clinical parameters in patient 1 who re ved her transplant from an identical twin, ap- from Fig. 2.

2. *Infection.* In one case examined once during acute pyelonephritis (case 3) the LMT was normal. A brief, transient reduction of the renal function, which developed in patient 12 during a septic episode, was associated with inhibition of LMT in three consecutive determinations (Table VI).

3. *Recurrence of glomerulonephritis.* The course of the LMT in patient 20 who displayed symptoms of acute glomerulonephritis four months after transplantation, appears from Fig. 3. The diagnosis was established by conventional and by immunofluorescent microscopic examination. LMT was inhibited in six, and normal in three examinations. The latter were all performed at a time when high-dose corticoid therapy was given (Fig. 3).

4. *Ischaemic damage.* The mean value of 15 LMT examinations in six patients (nos. 15 17 18, 24, 26 and 32) was normal (MI ± S.D = 1.00 ± 0.10) during transient anuria due to ischaemic damage to the graft.

DISCUSSION

With the method employed an antigen-induced inhibition of the leucocyte migration is an expression of cellular hypersensitivity (1 15).

After kidney transplantation it was neither possible to demonstrate organ-specific, antirenal cellular hypersensitivity in eight patients with an uneventful clinical course nor in the remaining 26 patients during uneventful periods.

During clinical episodes with reduced renal function it was possible in several cases to demonstrate an inhibition of the LMT possibly indicating a state of hypersensitivity developed in response to release of kidney-specific, antigenic material from disintegrating kidney cells. Most constantly the LMT was inhibited during acute rejections, viz. in ten out of 15 rejection episodes examined (Table V). The lack of inhibition in the LMT in some of the rejecting patients could possibly be due to the high-dose glucocorticoid therapy given at the time of examination and on the days immediately before, since it has been demonstrated that glucocorticoids can normalize a pathological LMT (3). On the other hand the LMT remained normal in two patients with a chronic rejection syndrome. A renal biopsy in one of

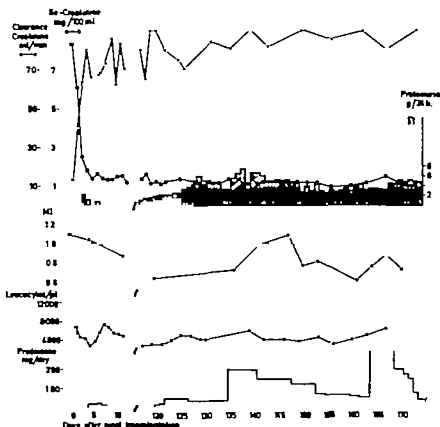


Fig. 3. Leucocyte migration test (LMT) and the clinical course in patient 20, who had recurrence of his original disease in the transplant (glomerulonephritis) four months after the transplantation.

these (no. 14) showed fibrosis, but no increased cellular infiltration.

It is assumed that inhibition of the LMT with pooled, normal kidney extract as antigen indicates the existence of organ-specific, antirenal cellular hypersensitivity and is less probably an expression of hypersensitivity to transplantation antigens of the kidney graft. This assumption appears to be valid for the following reasons: (i) unrelated tissue extracts did not induce inhibition in the LMT although they may be supposed to contain transplantation antigens in quantities comparable to that of kidney parenchyma, (ii) foetal kidney extract did not induce lymphocyte transformation in suspension cultures (18) indicating that, although the kidney extract might very well contain transplantation antigens, these were not present in active or available form (iii) organ-specific, antirenal cellular hypersensitivity is manifested as an LMT inhibitory effect of normal kidney homogenate in patients with sub-acute glomerulonephritis (2).

LMT was also inhibited in other episodes than rejection, i.e. in two cases of ureteral leakage (Table VI). These reactions, however might be

Table VI. *Leucocyte migration test (LMT) with extract of foetal kidney in seven patients during a periodic reduction of renal function not caused by rejection*

Pat. no.	Complication	Day(s) of complication	LMT
1	Leakage of ureter	12 17	0.57 0.75 0.70
3	Leakage of ureter	4	0.90
3	Leakage of ureter	28	0.93
3	Acute pyelonephritis	73–80	1.05
12	Sepsis	272 276	0.62, 0.78 0.79
13	Leakage of ureter	7–8	1.05
21	Leakage of ureter	20–27	0.77
27	Leakage of ureter	11	0.97
33	Leakage of ureter	6–14	1.13

caused by a rejection process with preferential localization to the ureter (11), and the LMT might again be considered expressive of transplantation-specific cellular reactivity This assumption is, however less probable because one of the patients with positive LMT had received a transplant from her identical-twin sister

In terms of organ-specific reactivity a more pronounced cell destruction could explain that the LMT was inhibited only in the two patients who had a protracted clinical development of their ureteral leakage with associated, slow reduction of the renal function, in contradistinction to the five cases with normal LMT who had acute clinical symptoms and received operative treatment on the same day

In one patient (no 20) who developed acute glomerulonephritis four months after the transplantation the LMT was followed through about two months (Fig. 3). Pronounced inhibition was demonstrated at the beginning of the disease and in periods when the glucocorticoid dose was moderate (i.e. lower than 50 mg prednisone per day) When the dose was increased the LMT became normal.

During initial anuria caused by ischaemic dam to the donor kidney the LMT was normal. biopsies, including patients nos. 15 24 d 33 showed pronounced destruction of the tubular epithelium, but no cellular infiltration of the parenchyma. The lack of inhibition in the LMT is in accordance with the findings in acute tubulo-interstitial nephropathy (3). It would be in accordance with the present findings if the development of organ-specific, antirenal cellular hypersensitivity in response to release of antigenic components from disintegrating kidney cells presupposes a contemporary or preceding lymphocyte infiltration of the renal parenchyma.

The specific alteration of reactivity occurred in all cases simultaneously with clinical signs and symptoms. This fast appearance of hypersensitivity could mean either (i) that it was the result of a booster effect of the antigen, which must then be supposed to have previously exerted antigenic activity in the same organism or (ii) that the cellular hypersensitivity demonstrated corresponds to the type of cellular hypersensitivity which is developed in the early phase of the immune response (*Jones-mote type, transient type*) and is demonstrated as early as 24 hours after the antigenic stimulus. In microbial hypersensitivity this early phase of cellular hypersensitivity is readily demonstrable with the LMT (17). Finally it has to be considered whether the LMT in the present modification, especially on account of the heterogeneity of the antigen preparation, may not be insensitive to low grade deviations from normal reactivity

Since the LMT with foetal kidney extract as antigen is positive during episodes of rejection as well as during other complications, the LMT with the present technique does not yield information of practical clinical value in the differential diagnosis of conditions with reduced renal function after kidney transplantation.

ACKNOWLEDGEMENT

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RENAL TRANSPLANTATION AND MURAMIDASE ACTIVITY

Urinary Muramidase as Indicato of Tubular Damage in Patients with Renal Transplants

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Abstract. Plasma and urinary muramidase activities have been measured in 26 patients with renal allogenic transplants. It was found that increased urinary muramidase rather accurately mirrored post-transplantation events associated with tubular damage, i.e. rejection, tubular ischemia, and leakage from the ureter. An increase in urinary muramidase sometimes preceded clinical diagnosis. There was no correlation between urinary muramidase activity and such nephrological parameters as proteinuria and creatinine clearance, when creatinine clearance was not grossly impaired. It is suggested that measurements of urinary muramidase may be of value in the clinical management of patients with renal transplants.

The bacteriolytic enzyme muramidase (lysozyme) is a strongly basic protein (isoelectric pH 10–11) of low molecular weight (14 000). The enzyme was discovered and described by Alexander Fleming who found it to be lytic largely to apathogenic bacteria (5 6, 7) but except for this the physiological significance of the protein is unknown. Muramidase exists in many tissues and secretions; it is *found* also in blood plasma, probably largely due to death of muramidase-containing cells of the blood, i.e. neutrophilic granulocytes and monocytes (3, 4). Normally the urine contains trace amounts only of muramidase (11 12, 18).

Lately new interest in this enzyme has been evoked as a diagnostic tool in hematology (raised plasma activity in certain leukemias and leukopenias due to increased turnover of neutrophilic granulocytes and monocytes) and nephrology In nephrology increased amounts of muramidase in the urine have been reported repeatedly mostly in patients with renal tubular defects, suggesting that in patients with a normal production of muramidase urinary muramidase may be an important indicator of tubular injury ("tubular proteinuria") (11 14, 18).

The significance of muramidase determinations in patients with renal transplants has been reported by Noble et al. (14) and by Shehadeh et al (19). The aim of the present work was to examine, in a large clinical material of patients with renal transplants, the significance of plasma and urinary muramidase as indicators of posttransplantation events, especially rejection.

MATERIAL AND METHODS

Twenty-six patients with renal allogenic transplants were studied. Table I summarizes relevant clinical information about these patients. Histocompatibility testing was carried out as described by Kissmeyer-Nielsen and Kjerbye (13). In the histocompatibility ranking the following terminology was used. *A match*, no incompatibilities between donor and recipient; *B match*, one minor incompatibility between donor and recipient; *C match*, one major incompatibility between donor and recipient; and *D match*, two major incompatibilities between donor and recipient. Impending rejection was diagnosed on the basis of the usual clinical signs (20)· the diagnosis of ischemic damage to the donor kidney was confirmed by renal biopsy in all patients with initial anuria; the diagnosis of ureteric leakage was established by pyelography and confirmed at operation in those cases of leakage where surgical intervention was deemed necessary

Muramidase activity in blood plasma and urine was determined by the lyso-plate method of Osserman and Lawlor (15). The normal value for plasma activity in our laboratory is 104 µg/ml ±53 (1 S.D.) (9), and for urinary muramidase less than 3 mg/24 h, based on examination of 25 patients with no hematological or renal

Table I. *Clinical data of transplanted patients*

Pat. no.	Age (y.)	Sex	Primary kidney disease	Donor	Match
1	44	♂	Chronic glomerulonephritis	Sister	A
2	32	♂	Polycystic kidney	Mother	C
3	34	♀	Chronic pyelonephritis	Mother	C
4	44	♂	Chronic pyelonephritis	Mother	C
5	23	♀	Chronic glomerulonephritis	Mother	D
6	21	♀	Chronic glomerulonephritis	Mother	C
7	40	♀	Subacute glomerulonephritis	Mother	A
8	46	♀	Chronic pyelonephritis	Necro	C
9	29	♂	Chronic glomerulonephritis	Brother	A
10	17	♀	Traumatic kidney injury	Necro	B
11	59	♂	Chronic glomerulonephritis	Necro	B
12	52	♂	Chronic pyelonephritis	Necro	B
13	21	♂	Chronic glomerulonephritis	Brother	A
14	25	♀	Chronic glomerulonephritis	Brother	A
15	33	♂	Chronic pyelonephritis	Necro	D
16	57	♂	Chronic pyelonephritis	Necro	B
17	40	♀	Chronic pyelonephritis	Necro	C
18	42	♀	Chronic pyelonephritis	Necro	C
19	46	♀	Chronic pyelonephritis	Necro	C
20	51	♀	Chronic pyelonephritis	Brother	A
21	48	♂	Chronic pyelonephritis	Necro	C
22	29	♂	Chronic glomerulonephritis	Brother	C
23	26	♀	Chronic pyelonephritis	Necro	B
24	44	♀	Chronic pyelonephritis	Necro	D
25	48	♀	Polycystic kidney	Necro	C
26	53	♂	Chronic glomerulonephritis	Sister	A

…e II. *Urinary muramidase in 12 patients with un…cated post-operative course after renal transplantation*

Pat. no.	Creatinine clearance normalized on day[a]	Base level of urinary muramidase reached on day	Urinary muramidase at base level[b] (mg/24 h)
1	2	2	6±3
2	11	7	5±3
3	7	7	5±3
4	21	13	10±1
5	13	13	13±1
10	17	16	6±5
12	4	5	37±27
13	3	6	11±13
18	3	3	10±13
21	6	6	3±3
22	3	6	4±9
23	3	2	13±22
		Mean	11±8
		Mean (excl. of pats. 12, 13 and 23)	7±4
		(Normal value	< 5 mg/24 h)

[a] Creatinine clearance exceeding 60 ml/min.
[b] Mean ± 1 S.D.

disease. As previously described (9), hen egg white muramidase is used as reference standard in our laboratory. With this standard muramidase activities are about ten times those found when human muramidase is used as a reference (10, 15). Plasma muramidase was measured three times weekly, urinary muramidase three times weekly at the beginning of the study, later daily. Measurements were carried out on 24-hour sample of the urine without addition of preservatives, samples were kept at room temperature until analysis.

The patients were studied during the immediate post-transplantation period, i.e. usually over a two to three week period.

RESULTS

The post-transplantation course of the 26 patients in the period of observation can be divided into four groups: 1) no complications 2) rejection episodes 3) ischemic damage to the donor kidney and 4) leakage of ureter.

1. Urinary muramidase activities of the 12 patients with uneventful post-operative courses are summarized in Table II. Fig. 1 shows the post-operative development in plasma and urinary muramidase activities of a typical patient. Immediately after transplantation plasma and uri-

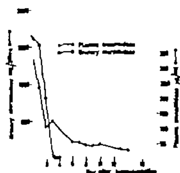


Fig 1 Plasma and urinary muramidase activities in a patient ... th successful post-transplantation course (pat. 22).

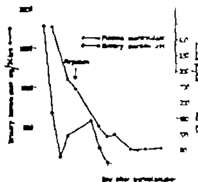


Fig ... Plasma and urinary muramid... patient ... ith rejection episode (p... 16

...nary muramidase activities were extremely high, plasma activities being at the high pre-transplantation level. Both decreased rapidly plasma muramidase becoming completely normalized, and urinary muramidase reaching a low near normal level. As a rule urinary muramidase remained at the low "base level" but three patients (pats. 1., 13 and 23) showed minor elevations from this level (maximal urinary muramidase 90, 35 and 75 mg/24 h, respectively). The "base level" of urinary muramidase was somewhat higher than normal urinary muramidase levels (Table II).

2. Table III shows urinary muramidase in the seven patients with rejection episodes. It is seen that all rejection episodes were accompanied or preceded by elevations in urinary muramidase activity. Plasma muramidase was not a good indicator of rejection. Fig. 2 shows plasma and urinary muramidase in a typical patient with rejection.

Table III. *Urinary muramidase in seven patients with rejection*

Pat. no.	Day(s) of clinical rejection	Day(s) of rise in urinary muramidase	Maximal urinary muramidase (mg/24 h)
7	10–18	8–15	81
11	13–15	14–25	63
11	64 (chronic)	64	32
15	3–5	3–4	990
16	5–7	4–9	569
17	37 (chronic)	37	240
24	6–21	7 11	210
26	17–21	19–21	41

3. Four patients had ischem... transplanted kidney (pats. 8 ... of these patients had elevate... midase when urinary output ... initial anuria (maximal urin... tivities 1 090 618 137 ... respectively). Fig. 3 demons... nary muramidase in such ...

4. Data from the seven p... due to ureteric leakage are sh... and plasma and urinary muram... such patient is demonstrated in Fig. ... that all episodes of ureteric leakage w... preceded or accompanied by elevated muramidase activities.

Table V shows the correlation coeff... plasma and urinary muramidase vers... c... nine clearance and proteinuria. It is se... that there was no correlation between these parameters.



Fig. 3. Plasma and urinary muramidase activities in patient with ischemic damage to the donor kidney. Due to anuria total urinary muramidase activity was low initially. With increasing diuresis total urinary muramidase activity rose concomitantly. H. D indicates hemodialysis (pat. 17).

Table IV *Urinary muramidase in patients with leakage from the ureter*

Pat. no.	Day of clinical detection of ureteric leakage	Day of rise in urinary muramidase	Maximal urinary muramidase (mg/24 h)
6	7	6	712
8	4	—	—
9	12	10	168
14	14	6	439
16	14	13	74
20	11	11	88
26	6	6	72

Table V *Correlation coefficients of plasma and urinary muramidase versus proteinuria and creatinine clearance*

N = number of paired observations

	Proteinuria	Creatinine clearance
Urinary muramidase	0.00 ($N = 411$)	0.11 ($N = 398$)
Plasma muramidase	0.04 ($N = 239$)	0.28 ($N = 232$)

DISCUSSION

Plasma and urinary muramidase activities depend upon a balance between production and elimination. Thus, whereas normally trace amounts of muramidase only are found in the urine, significant muramidasuria appears when plasma activity exceeds the normal plasma level by approximately three times, suggesting a renal threshold (12). In the kidney muramidase is believed to be filtered in the glomeruli (cf. the low molecular weight) and catabolized in the proximal tubules. Thus, in animal studies with graded damage, decreased tubular reabsorption muramidasuria were seen after poisoning with sodium chromate which damages the proximal tubules (2). Damage to the tubules by cadmium also produced muramidasuria (1) In contrast, glomerular injury produced only very slight muramidasuria (18). The correlation between tubular damage and muramidasuria has been confirmed in large clinical materials where significant muramidasuria was seen in patients with tubular defects (e.g. the Fanconi syndrome) whereas other types of renal disease very rarely were associated with increased urinary muramidase activity (11 12, 16, 18)

Urinary excretion of muramidase after renal transplantation has been studied in both dogs and humans (14 19). In these studies rejection episodes were associated with the development of significant muramidasuria, and the authors found increased urinary muramidase to be a reliable sign of rejection. There was, however, in these reports, no mention of whether other post-transplantation events were also associated with increased urinary muramidase.

This study has confirmed that urinary muramidase rises in conjunction with rejection episodes, sometimes before clinical signs are recorded. This finding agrees well with the fact that tubular damage is a prominent factor in rejection (17). The present study has, however not confirmed that urinary muramidase is a specific indicator of rejection since urinary muramidase also rose in other post-transplantation events associated with tubular damage (ischemic injury and leakage from the ureter). The fact that urinary muramidase rose in cases with ureteric leakage could be an indication of tubular damage caused by renal stasis (21)· another possible explanation could be, however that ureteric leakage developed because of ureteric rejection, and that the increased urinary muramidase activity reflected concomitant renal rejection with tubular impairment.

The development of plasma and urinary muramidase activities in the patients with normal post transplantation courses may provide information about tubular function in the transplanted kidney

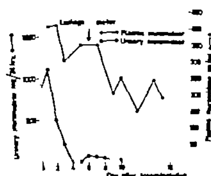


Fig. 4. Plasma and urinary muramidase activities in a patient with uroplania due to ureteric leakage (pat. 26).

Thus the early fast normalization of the usually very high pre-transplantation plasma muramidase activity and the precipitous fall in urinary muramidase towards normal values immediately after transplantation indicate that the tubules function rather well from the beginning. The fact, however that urinary muramidase activities at "base level" were somewhat higher than normal values may indicate that, although grossly normal, a minor degree of tubular impairment remains after transplantation. Other studies have indicated tubular dysfunction of transplanted kidneys (8)

The present findings that urinary muramidase rather accurately mirrors post-transplantation events associated with tubular damage, sometimes providing an early warning before clinical diagnosis, and the fact that urinary muramidase activities are not correlated with such nephrological parameters as proteinuria and creatinine clearance indicates that measurements of urinary muramidase activity may be of some value in the clinical management of patients with renal transplants.

ACKNOWLEDGEMENTS

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FAMILIAL LECITHIN-CHOLESTEROL ACYLTRANSFERASE DEFICIENCY

Study of Two New Patients and their Close Relatives

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Abstract. A family with two patients, brother and sister, afflicted with familial lecithin:cholesterol acyltransferase deficiency is described. The patients had corneal infiltration, normochromic anemia, and proteinuria. No lecithin:cholesterol acyltransferase activity could be demonstrated in their plasma. They had high plasma concentration of unesterified cholesterol, lecithin and triglyceride, and low concentration of cholesteryl ester, which acyl groups were mainly saturated and mono-unsaturated. Paper electrophoresis of plasma lipoproteins showed absence or highly reduced levels of α- and pre-β-lipoproteins. The erythrocytes contained increased amounts of cholesterol and lecithin, and decreased amounts of sphingomyelin and phosphatidylethanolamine. Post-heparin lipolytic activity tested in one of the patients, showed values within the normal range. No relevant abnormalities were found in the parents of the patients or in their only sib, a healthy female.

Lecithin:cholesterol acyltransferase (LCAT) is normally present in human plasma. The enzyme apparently plays a key role in the formation of plasma cholesteryl ester (6, 8). Recently a familial disease was discovered in which the plasma LCAT is lacking (3 7 8). Until now five such patients are known. The disease is characterized by corneal opacity anemia, proteinuria, and by markedly reduced levels of plasma cholesteryl ester and lysolecithin, high levels of plasma unesterified cholesterol and lecithin, and by absence or very low levels of LCAT activity

The present paper describes two more patients with familial LCAT deficiency The parents are from an isolated community on the West coast of Norway To the best of our knowledge they are not related. They had three children, the two patients and a healthy daughter These five members of the family have been studied.

CASE REPORTS

Case 1

A female born in 1926. She has been married for several years but has not been pregnant. She has previously been healthy especially having no history of hepatitis or nephritis. Proteinuria and anemia have been present ever since they were discovered in 1958. She was hospitalized for a short period in 1965 due to menorrhagia. In 1966 she was admitted to the Medical Department, Kristiansand Hospital, Kristiansand, Norway because of proteinuria, anemia and a slightly elevated blood pressure, when she weighed 90 kg and was 165 cm tall. She was pale; no edema, exanthema or xanthoma were observed. Corneal opacity was seen in both eyes. The infiltration was diffusely located, but most concentrated in the periphery where it resembled arcus lipoides. The tonsils and lymph nodes were normal, and physical examination of lung, heart and abdomen revealed no abnormalities. Liver, spleen and kidneys were not palpable. The reflexes were normal. Blood pressure varied between 200/115 and 160/90 mmHg. ECG was normal, and X-ray of chest and intravenous renography showed normal pictures. She had slight proteinuria (0.5–1 75 mg protein/ml of urine), and some hyaline casts and erythrocytes were present in the urine sediment. She had normochromic anemia (8.9–10.9 g Hb/100 ml). The amounts of circulating thrombocytes and leucocytes were normal, and differential counts revealed no abnormalities. Serum protein concentration was 6.1 g 100 ml (albumin 2.4, α_1-globulin 0.7 α_2-globulin 0.7 β-globulin 0.9 and γ-globulin 1.4 g/100 ml). The concentration of uric acid in plasma was 8.8 mg/100 ml. Normal values in plasma were found for urea, creatinine, sodium, potassium, chloride, phosphate, calcium, glutamic, pyruvic and glutamic oxaloacetic transaminase, and vitamin B_{12}. Fasting blood sugar, urinary excretion of catecholamines, fecal content of lipids and basal metabolic rate were normal.

She had hyperlipemia with increased amounts of triglyceride, phospholipids and total cholesterol in plasma. The ratio of esterified to unesterified cholesterol in plasma was abnormally low At that time (February 1966) no explanation was found for these plasma lipid abnormali-

Table I. *Plasma lipid fractions in two patients with familial lecithin cholesterol acyltransferase deficiency*

	Case 1	Case 2	Normal range
Unesterified cholesterol (mg/100 ml)	320	320	50–90
Cholesteryl ester (mg/100 ml)	40	30	120–210
Triglyceride (mg/100 ml)	1900	434	20–150
Phospholipids (mg/100 ml)	554	374	200–300
Lecithin (% of total phospholipids)	85	82	69–78
Lysolecithin (% of total phospholipids)	2.0	2.8	3.7–9.3
Cephalins (% of total phospholipids)	3.4	7.4	2.9–5.6
Sphingomyelin (% of total phospholipids)	8.8	7.8	12.6–21.8

ties. Since 1966 she has been controlled regularly and no progress in her anaemia or proteinuria has been observed.

Case 2

A male born in 1932. He is unmarried and has no children. Since early childhood he has had slight atrophy f the left arm and hand (birth trauma?). He has no ory of hepatitis or nephritis. Proteinuria was detected 1950. In 1958 he was tonsillectomized due to recurrent llitis. A routine examination in 1962 revealed anemia and proteinuria. In 1963 he was admitted to the Medical Department B, Rikshospitalet, Oslo, Norway when he weighed 88.5 kg and was 186 cm tall. He had no edema, exanthema or xanthoma. Corneal infiltration was seen in both eyes, most pronounced in the periphery. He had a slight atrophy of left arm and hand. A further physical examination revealed no abnormalities. Blood pressure was 135/70 mmHg. ECG was normal and X-ray of chest and intravenous renography showed normal pictures. He had proteinuria (1.2 mg protein/ml urine), and some casts and erythrocytes were found in the urine sediment. He had normochromic anemia (11.2 g Hb/100 ml). The amounts of circulating thrombocytes and leucocytes were normal, and differential counts revealed no abnormalities. Serum protein concentration was 6.4 g/100 ml (albumin 3.6, α_1-globulin 0.4, α_2-globulin 0.6, β-globulin 0.7 and γ-globulin 1.1 g/100 ml). He had hyperlipemia. The serum lipid concentration was (mg/100 ml): total cholesterol 300–340, phospholipids 520–560, and total lipids 1950–2900. The concentration of uric acid in plasma was 10.6 mg/100 ml. Normal values in plasma were found for urea, creatinine, bilirubin, sodium, potassium, chloride, calcium, phosphate, alkaline phosphatase, and glutamic oxaloacetic transaminase. Fasting blood sugar and and oral glucose tolerance test were normal.

He has felt healthy since 1963 but on examination proteinuria and anemia have always been present.

THE FAMILY

Father of the patients. Born in 1883. He had an eye injury as a boy and has since been blind in his right eye. He has been in good health until recently. He now has a slight heart failure and flimmer arythmia. His right eye is amaurotic with a scarified cornea. He has an arcus senilis in his left cornea, but no infiltration like that of the patients is present. Determination of serum electrolytes, urea, creatinine and lipids revealed no abnormalities. The amounts of plasma α-lipoprotein and the ratio of esterified to unesterified cholesterol in serum were normal.

Mother of the patients. Born in 1899. Healthy until 1959 when she suffered from a hemiplegia due to hypertension. On physical examination a slight hemiparesis was found. B.P. was 200/100 mmHg. Determination of serum electrolytes, urea, creatinine and lipids revealed no abnormalities. Plasma α-lipoprotein was present in normal amounts, and the ratio of esterified to unesterified cholesterol in serum was normal.

Unmarried *sister* of the patients. Born in 1928. She was tonsillectomized in 1951. In the last trimester of her only pregnancy she had proteinuria, which disappeared after the delivery. Physical examination revealed no abnormalities. Serum electrolytes, urea, creatinine and lipids were normal. Plasma α-lipoproteins was present in normal amounts, and the ratio of esterified to unesterified cholesterol in plasma was normal.

SPECIAL STUDIES

Cases 1 and 2 had several symptoms and findings suggestive of familial LCAT deficiency. The blood of these patients was therefore studied in greater detail. The methods used have been published previously (5–8).

Plasma lipids. Table I shows that the patients had very low levels of cholesteryl ester in plasma, whereas the concentration of unesterified cholesterol was abnormally high. The plasma triglyceride and phospholipid were high, and the pattern of phospholipid was abnormal, as the relative amount of lecithin was high and that of sphingomyelin and lysolecithin low.

Lecithin:cholesterol acyltransferase. No LCAT activity could be demonstrated in the plasma of the patients, whereas normal activities were found in plasma from their parents and healthy sister.

Plasma lipoproteins. Paper electrophoresis of plasma from the patients revealed the same lipoprotein pattern as found in all other patients with LCAT deficiency previously studied. Thus the lipoproteins migrated only to the β-lipoprotein region, with an abundant trailing towards the application site. No α-lipoprotein or pre-β-lipoprotein could be demonstrated. Upon immunoelectrophoresis against anti-α-lipoprotein serum (Behringwerke) a faint precipitin line, located more anodically than the normal α-lipoprotein precipitin line, was found. This suggested that the patients had reduced levels of α-lipoprotein and that their α-lipoprotein had an abnormally slow electrophoretic mobility (10).

The acyl patterns of plasma cholesteryl ester from the two patients are shown in Table II. As in the other patients with LCAT deficiency the fraction of saturated and monounsaturated acyl groups is increased, and that of poly-unsaturated acyl groups decreased compared to normal findings (9).

Erythrocyte lipids. The cholesterol and lecithin contents of the red cells from cases 1 and 2 were almost twice as high as in normal erythrocytes (Table III). The total phospholipid concentration, however, was not increased, as the amount of sphingomyelin and phosphatidylethanolamine was decreased.

Lipoprotein lipase. Case 1 received 1 mg of heparin per kg body weight i. and the lipolytic activity of her plasma was tested according to Boberg and Carlson (1), with the only modification that the substrate suspension was homogenized with Branson sonifier before the plasma was added. *Fig. 1 shows the lipolytic activity* and the concentration of free fatty acids in the plasma of the patient after the heparin injection. The peak level of lipolytic activity was within the normal range found in our laboratory (0.35–0.70 mEq free fatty acid/l/min).

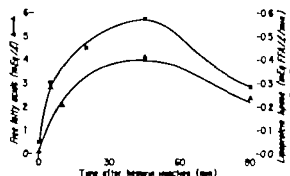


Fig. 1. Activity of plasma lipoprotein lipase and concentration of plasma free fatty acids in case 1 after intravenous injection of heparin (1 mg/kg body weight).

DISCUSSION

Most of the findings in the two patients are in good agreement with those obtained in previous studies of patients with familial LCAT deficiency (3, 5, 7, 8, 11). Thus the following abnormalities seem to characterize the disease.

The patients have corneal infiltration, normochromic anemia, proteinuria, and absence or near absence of plasma LCAT activity. Furthermore, they have a high plasma concentration of unesterified cholesterol, lecithin and triglyceride, and low concentration of cholesteryl ester which acyl pattern is characterized by high fractions of saturated and mono-unsaturated acyl groups. Paper electrophoresis of the plasma lipoproteins shows absence or highly reduced amounts of α- and pre-β-lipoprotein. Finally the erythrocytes contained increased amounts of *cholesterol* and *lecithin* and decreased amounts of sphingomyelin and phosphatidylethanolamine.

Our two patients had increased amounts of plasma uric acid. This has also been found in another patient (A. R., ref. 3). This abnormality however is not typical of familial LCAT deficiency as three other patients had normal plasma uric acid level (7, 11).

In the only patient previously tested the plasma post-heparin lipolytic activity was abnormally low (7). In case 1 in this study however we found normal amounts of lipolytic activity after injection of 1 mg/kg b. w. heparin intravenously. In another patient tested we have also found that the peak of lipolytic activity after heparin injection was within the normal range (unpublished observation). Thus it is difficult to explain the hypertriglyceridemia in the patients on the basis of reduced lipoprotein lipase. It may however partly be a consequence of the decreased amounts of α-lipo-

Table II. *Plasma cholesteryl ester acyl groups*

	12:0[a]	14:0	16:0	16:1	18:0	18:1	18:2	20:4
Case 1	1.4	2.9	27.4	6.7	7.2	35.1	16.9	trace
Case 2	—	1.6	24.4	6.3	8.8	42.8	12.5	3.7
Normal	0.3	1.2	12.4	6.1	2.4	18.4	47.8	5.3

The fatty acids designated by chain length: number of double bonds. Values expressed as per cent of total fatty acid methyl esters.

Table III. *Erythrocyte lipids*

	Case 1	Case 2	Normal[a]
Cholesterol 10^{-10} mg/cell)	1.9	1.8	1.1
Total lipid phosphorus (10^{-11} mg/cell)	1.2	1.0	1.0
Lecithin (% of total phospholipids)	53.2	53.7	27.4
Sphingomyelin (% of total phospholipids)	14.6	11.0	25.9
Phosphatidylethanolamine (% of total phospholipids)	17.6	19.5	29.0
Phosphatidylserine (% of total phospholipids)	12.6	10.0	14.0
Phosphatidylinositol (of total phospholipids)	0.9	3.9	1.6
Lysolecithin (% of total phospholipids)	1.1	1.9	2.1

Data from ref. 4 and 5

protein in plasma, as it has been postulated that α-lipoprotein has a role in the exodus of triglyceride from plasma (2).

The family described in the present report is from the Western part of Norway not so far away from the place where a family with three afflicted female patients is living (11). We have no evidence of a relationship between the two families.

Studies of the, until now seven known patients with familial LCAT deficiency and their close relatives have not clarified the mode of inheritance of the disease. It is, however probably autosomal recessive, as both sexes are afflicted, and as neither parents nor children of the patients have the disease (7 11). In none of the families have any relevant abnormalities been found in the presumptive heterozygotes.

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COMPARISON BETWEEN VARIOUS METHODS USED TO CONTROL DICUMAROL THERAPY

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Abstract. A comparison has been made between the following methods used to control dicumarol therapy: P & P (human thromboplastin), Simplastin A (rabbit thromboplastin), thrombotest (bovine thromboplastin), Quick's original method and Lehman's modification of this method (human and rabbit thromboplastin). It is shown that human and bovine but not rabbit thromboplastin is sensitive to the inhibitor formed during dicumarol therapy. In spite of this it is possible to use Biggs and Denson ratio method (1) to compare the "prothrombin" determination methods. By means of linear regression analyses the therapeutic level of the different methods are compared. In the discussion it is stated that methods of this kind never give a true picture of the dicumarol effect on the hemostasis. They must be regarded as indicators.

The use of different control methods is one of the reasons why varying results are obtained with dicumarol therapy. The effect of the therapy depends on its intensity, which should be as high as possible considering the risk of bleeding.

The varying results obtained by different investigators are at least partly due to the fact that the therapeutic level has varied. It is the aim of cooperating international research teams to find ways of comparing the control methods with each other and to find standards which do not change during storage.

METHODS

Coagulation methods

The P & P method as originally described by Owren and Aas (3) was used. The standardization was made using commercially normal plasma (Verify normal, Warner-Chilcott, Morris Plains, N.J., USA). A dilution, 1:10 of the reconstituted plasma, was first made with a dilution fluid of veronal-buffered physiological saline containing 11 mM sodium citrate (citrated buffer solution). This 1:10 dilution of normal plasma was further diluted 1:2, 1:4, 1:8 and 1:16. The dilution fluid for this dilution series was a 1:10 dilution of adsorbed bovine plasma in the same citrated buffer solution as is mentioned above. The standard curve was plotted on double logarithmic paper.

Quick's method

In a test tube 0.2 ml citrated plasma was mixed with 0.2 ml thromboplastin (human or rabbit) and recalcified with 0.2 ml $CaCl_2$ 0.025 M. The clotting time of the same commercially normal plasma as mentioned above was used as the normal clotting time of the system.

Quick–Lehman method

In a test tube 0.2 ml citrated whole blood was mixed with 0.2 ml thromboplastin (human or rabbit) and recalcified with 0.2 ml $CaCl_2$ 0.025 M. Quick–Lehman's index is: clotting time of normal blood/clotting time of patient's blood × 100. For instance, normal blood clotting time = 15 sec; patient's blood clotting time = 30 sec; index = 15/30 × 100 = 50%. In this investigation the normal blood clotting time was the mean of the clotting times of blood from ten presumably healthy persons.

Simplastin A, plasma method

Plasma was diluted 1:10 with the citrated buffer solution mentioned below. 0.1 ml of this dilution was mixed with 0.2 ml of the Simplastin A (Warner-Chilcott, Morris Plains, N.J., USA) reagent at 37°C. The clotting time was determined in the usual manner by tilting in a water bath at 37°C. The standard curve was made with the same dilutions as used in the standardization of the P & P test. This was done to ensure that the two methods were standardized in exactly the same manner. The standardization was tested with two artificially made defect plasmas (Verify abnormal I and II, Warner-Chilcott, Morris Plains, N.J. USA). Verify I gave 13% in the P & P test and 12.6% in Simplastin A. Verify II gave 5.1% in the P & P test and 3.6% in Simplastin A.

Simplastin A, whole blood method

0.3 ml of the citrated buffer solution was placed in a plastic cup. From the finger tip, 0.05 ml blood was col-

lected and added to the cup. When the test was performed, 0.1 ml of this blood dilution was added to 0.2 ml Simplastin A reagent. The test was made in the same manner as with plasma dilution. The result was read on the same standard graph as for plasma.

Thrombotest

This method was carried out exactly as described by the manufacturer (*Nyegaard & Co., Oslo Norway*). The investigation was made on citrated whole blood collected as described below The result was read on the correlation graph made by the manufacturer T read values above 100% the standard graph was extrapolated.

Collection of blood

Venous blood (4.5 ml) was collected from the antecubital vein by a 16-gauge silicone-treated needle into plastic syringe containing 0.5 ml 0.1 M sodium citrate.

Capillary blood was collected from the finger tip. Of the first blood drops, 0.05 ml was collected in glass pipette and added to the dilution fluid described below

Citrated buffer solution

Na-diethyl barbiturate 1.046 g, HCl 1-N 3.56 ml, Na-citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) 1.235 g, NaCl 7 740 g, Aq dest. ad 1 000 ml.

Statistical methods

Ordinary statistical methods (6) were used for the linear regression analyses.

Biggs and Denson's method for comparison between control methods for dicumarol therapy (1)

1 Blood samples from a group of dicumarol-treated patients were taken and analysed by the different methods to be compared.

2. For each of the methods the ratio between the patient clotting time and the normal clotting time for the method was calculated. For instance the normal clotting time of the method = 22 sec, the clotting time for the patient = 44 sec. The ratio is then 44/22 = 2.

3 One of the methods was chosen as standard method. The relation between the ratio of this method and the ratio of the other methods could now be determined either geometrically or statistically

4 It was now possible to calculate what certain ratio in the standard method corresponded to in the other methods.

5 Finally if desired, a standard graph for each of the methods, which corresponds to the standard graph of the standard method, can be constructed. In this work the comparison was made by statistical methods.

In the present work the equation of the straight line, i.e. $y = bx + L$, was used when particular ratio of one method was compared with that of another For instance, the regression analyses of the ratios of the P & P method and those of the Simplastin A method showed that the regression coefficient was 1 15 and the constant 0.11

Then $y = 1.15x + 0.11$

y = PP Simplastin A

In order to calculate what, for instance, 10% in P & P corresponds to in Simplastin A, it is necessary to calculate what is the ratio for 10% in P & P

The clotting time corresponding to 10% is 100 sec. The normal clotting time of the system (100%) is 26 sec. The ratio for 10% is then 100/26 = 3.84.

The ratio in Simplastin A which corresponds to this ratio in P & P is $x = (3.84 - 0.11)/1\ 15 = 3.24$.

The clotting time in Simplastin A which corresponds to the ratio 3.24 is 3.24 21 = 68 sec, because the normal clotting time (100%) in Simplastin A is 21 sec. Read on the standard graph for the Simplastin A method, 68 sec gives 14% i.e. 10% in P & P corresponds to 14% in Simplastin A.

MATERIAL

1 Patients on long-term dicumarol therapy
2 Patients beginning to receive dicumarol.
3. Normal persons not treated with dicumarol.
4 A few patients with parenchymatous liver disease and a decreased "prothrombin value.

RESULTS

Fig. 1 shows a comparison between the P & P test and the Simplastin A test. It was not possible to plot all the 237 pairs of analyses on dicumarol-treated patients. The cases plotted were picked consecutively from the start of the investigation. The calculation shown in Table I is based on all the analyses.

The figure shows that the values for Simplastin A are higher than for P & P in the range below about 50% in the P & P test. In the range from about 50% to about 100% however there is an equal distribution around the mean line.

Fig. 2 shows a comparison between P & P and thrombotest. In this case the P & P values were higher than the thrombotest values in the range below about 50% in P & P but equal in the range 50%–100%

Fig. 3 shows a comparison between the Simplastin A plasma method and the whole blood method. As can be seen, there is an equal distribution around the mean line in all ranges.

As two methods (P & P and Simplastin A), standardized with the same normal plasma dilutions, diverged when plasma from dicumarol-treated patients was tested, the conclusion must be that they did not measure exactly the same variables.

In the present investigation the hypothesis was that the P & P test, as well as thrombotest, were

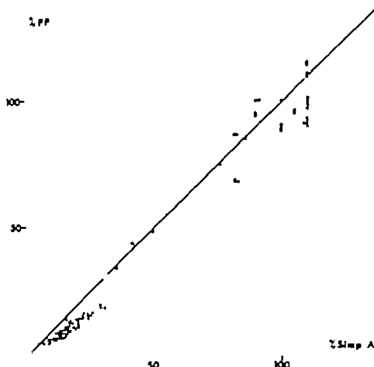


Fig. 1. Comparison between P & P and Simplastin A. ● dicumarol-treated patients; ○ normal persons or patients with parenchymatous liver disease.

sensitive to an inhibitor formed during dicumarol treatment, whereas Simplastin A was not sensitive to it (4). To test this hypothesis, the following experiments were made.

1. Human (used in the P & P test) and bovine (used in thrombotest) brain thromboplastin were produced as described by Owren and Aas (3).

2. Dilution series (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:126) with adsorbed bovine citrated plasma ("prothrombin"-free) were made with the following plasmas.

A. Plasma from dicumarol-treated patients (pooled from 10 patients).

B. Plasma from normal persons (pooled from 10 persons).

C. Plasma from a patient with parenchymatous liver disease.

3. On these three dilution series Quick's thromboplastin time was determined with (*a*) rabbit thromboplastin (Simplastin), undiluted and diluted 1:150 (*b*) human thromboplastin, undiluted and diluted 1:75 (*c*) bovine thromboplastin at an optimal concentration. (Bovine thromboplastin obviously contains inhibitors and has to be diluted to give the shortest possible clotting time.)

For technical reasons the dilution 1:2 was taken as a starting point when the results were plotted. Thus, what is called 1 in the figures is the dilution 1:2, 2 is the dilution 1:4 etc.

These kinds of experiments have been made by Hemker et al. (2) to show the existence of the inhibitor in dicumarol plasma.

Fig. 4 shows the results when rabbit thromboplastin was used. When plotted in this manner straight lines were obtained. As can be seen, the lines from dicumarol patients and normal persons meet on the ordinate, i.e. in $x = 0$ whether diluted or undiluted thromboplastin was used.

Fig. 5 shows the results when human thromboplastin was used. In this experiment the line from the dicumarol plasma dilutions and from the normal plasma dilutions did not meet on the

Table I. *Comparison between ratios for PP and Simplastin A*

	No. of pairs	Regression coefficient	Constant	Correlation coefficient
All pairs	237	1.19	−0.05	0.96
Ratio > 1.5	170	1.15	0.11	0.95
Ratio < 1.5	67	0.64	0.44	0.70

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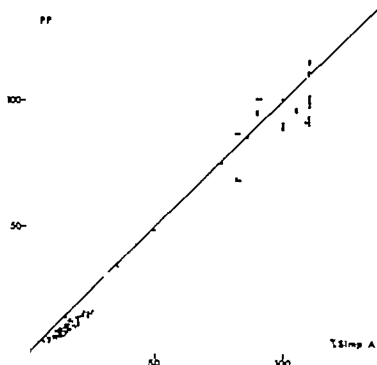


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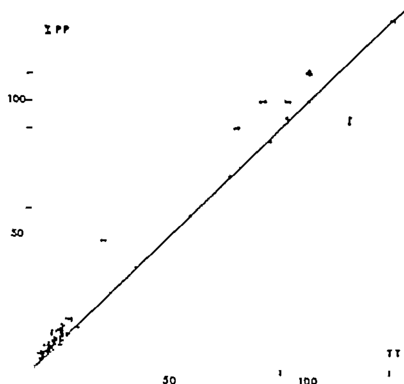


Fig. 2. Comparison between P & P and thrombotest. Symbols as in Fig. 1

ordinate. Instead, the lines meet at a point corresponding to about −2 on the abscissa. This is what would be expected if the dicumarol plasma contained an inhibitor.

Fig. 6 shows the results with bovine thromboplastin. In this case as well the lines meet at a point corresponding to about −2 on the abscissa.

Plasma dilutions from normal persons and from

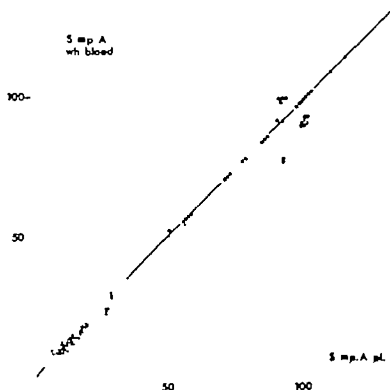


Fig. 3. Comparison between the Simplastin A plasma method and the whole blood method. Symbols as in Fig. 1.

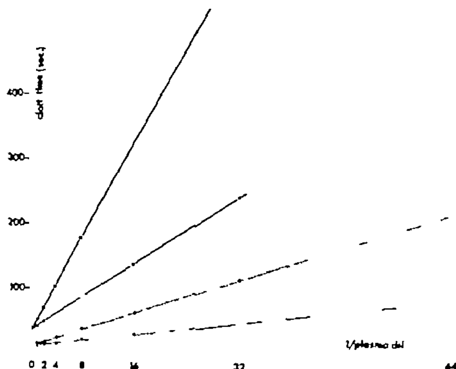


Fig. 4 Dilution series of plasma from dicoumarol-treated patients (O-O) and normal persons (● ●) tested with rabbit thromboplastin, undiluted (— —) or diluted 1:150 (—). Abscissa inverse of plasma dilution. Dilution fluid bovine citrated adsorbed plasma.

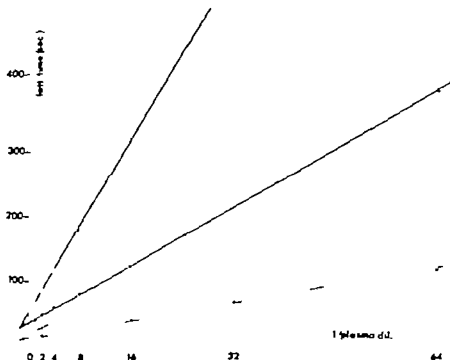


Fig. 5 The same plasma dilutions as in Fig. 4 tested with human thromboplastin undiluted (— · —) or diluted 1:75 (—).

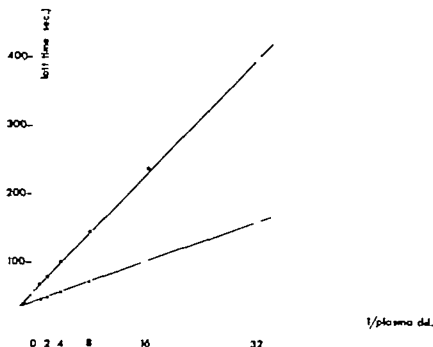


Fig. 6. The same plasma dilutions as in Fig. 4 tested with bovine thromboplastin of optimal activity

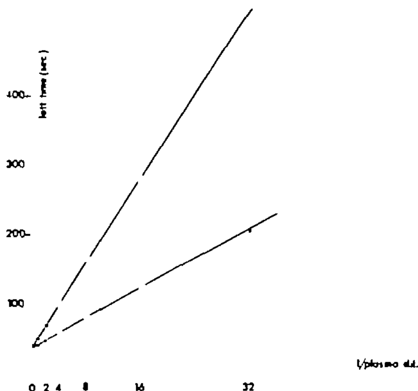


Fig. 7. Dilution series of plasma from patient with parenchymatous liver disease (O-O) and from normal persons (●-●) tested with human thromboplastin.

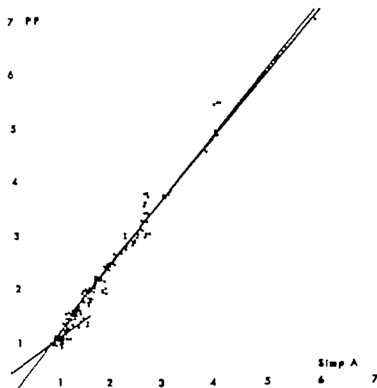


Fig. 8. Comparison between the ratios for P & P and Simplastin A. Figures on abscissa and ordinate are ratios. Dotted line indicates regression line for the whole population. Solid lines indicate regression lines when material divided into one group above and one below ratio 1.5 in P & P.

patients with a decreased prothrombin value due to parenchymatous liver disease, tested with human thromboplastin, are shown in Fig. 7. The lines again meet on the ordinate. These results confirm the findings of Hemker et al. (2) that there is an inhibitor present in dicumarol plasma, and show that rabbit thromboplastin probably is not sensitive to this inhibitor whereas human and bovine thromboplastin are sensitive to it.

Comparison between the coagulation methods using Biggs' ratio method

Fig. 8 shows a comparison between the ratios for P & P and Simplastin A. The figures for the experiment are given in Table I. The dotted line in the figure indicates the linear regression line for the whole population. From the results presented in Fig. 1 it is improbable that linear regression is the best model when the whole population of pairs is analyzed. Instead of trying other mathematical models it was considered natural to separate the material into two groups corresponding to those with values above and those with values below 50% in P & P. A P & P value of 50% corresponds to a ratio of 1.5. When the regression lines were calculated separately for these two groups (i.e. above and below the ratio 1.5 in P & P) the results were somewhat different. The solid lines in Fig. 8 represent the regression lines in the two populations. In this case the group with a ratio below 1.5 was comparatively small and did not considerably influence the regression lines of the population with a ratio above 1.5. It is clear however that if linear re-

Table II. *Comparison between therapeutic levels of five methods for control of dicumarol treatment*

	P & P Human thromboplastin	TT Bovine thromboplastin	Simplastin A. Rabbit thromboplastin	Quick–Lehmann. Human thromboplastin	Quick–Lehmann. Rabbit thromboplastin
Therapeutic level	10–25 %	7–18 %	14–34 %	35–55	57–72 %

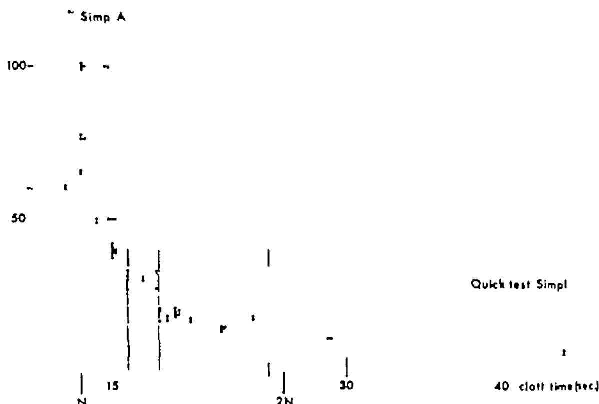


Fig. 9. Comparison between Simplastin A and Quick's method. Horizontal solid lines, therapeutic level of Simplastin A. Vertical lines (— — —), therapeutic level of Quick's method calculated by means of ratio method. Vertical lines ( ), therapeutic level as given by the manufacturer of the thromboplastin. N normal clotting time in Quick's method. 2 N twice normal clotting time.

gression is used, the comparison must be made in the range where the methods are to be used for measurement, i.e. in the therapeutic range. The correlation coefficient, as can be seen from Table I was very high.

Similar experiments have been made to show the correlation between P & P and thrombotest, thrombotest and Simplastin A P & P and Quick's method, both with human and rabbit thromboplastin and Quick–Lehman's method both with human and rabbit thromboplastin. In these calculations as well only values with ratios higher than 1.5 in P & P were considered.

The results were used to compare the therapeutic levels of the different methods. The P & P method was chosen as a standard method and the therapeutic level was set at 10–25%. Table II shows the results calculated on the basis of the relation between the ratios, as described under Methods. Observe the big difference in therapeutic level between Quick–Lehman's method when performed with human and with rabbit thromboplastin.

In Fig. 9 Simplastin A expressed in per cent is compared with Quick's method, using rabbit thromboplastin and expressed in seconds. The calculated therapeutic level for Quick's test does not correspond exactly to the therapeutic level given by the manufacturer. This illustrates what has been known for a long time, i.e. that the intensity of the treatment is higher in places where Quick's method with rabbit thromboplastin is used for control of dicumarol therapy. Twice the clotting time in this case falls below the lower limit as calculated from the therapeutic level when the P & P test is used.

DISCUSSION

It is obvious that the different methods used to control dicumarol therapy do not measure exactly the same variables. What qualities are desired in

a method to control dicumarol therapy? First of all the method must reflect the dicumarol effect. Today it is known that dicumarol decreases the synthesis of four known clotting factors and gives rise to the formation of one or more inhibitors. In the future it may very well be observed that the effect is still more complex. In my opinion the aim should therefore not be to elaborate on global tests which reflect all these effects. No matter how the tests are designed, it will not be possible to know how much one or the other factor influences the test in the single patient. Such methods can never give a true picture of the coagulation potential. When citrate is added, the test solution diluted, tissue thromboplastin added etc., it is no longer a matter of true physiology If the aim is to get as total a picture of the coagulation potential as possible, the whole blood clotting time should be preferred. In fact it has been used for this purpose, but has been rejected by most investigators because it is not sensitive enough.

Probably one of the variables influenced by dicumarol can be used equally well as a measure.

Regardless of how the method is designed, the only way to determine the therapeutic range is to correlate the figures from measurements with antithrombotic effect and bleeding episodes in big clinical materials.

International cooperative studies must aim at establishing uniform control methods. Probably this will take many years. In the meantime Biggs ratio method seems to give us an opportunity to compare the therapeutic levels of different methods. In this way a meaningful exchange of experience can take place.

If this method for comparison is accepted, the question of standardization is still not solved. Up to now lyophylized pooled normal plasma or "standard" preparations of thromboplastin (5) have been used as standards. International agreement on what kind of standards we are to use is necessary

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TERTIARY HYPERPARATHYROIDISM

Report of a Case

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Abstract. A 30-year-old woman suffered from anorexia nervosa from the age of 14, and vomiting from the age of 18. During recent years she has suffered from renal failure, and on admission to our hospital she had the biochemical changes of "primary" hyperparathyroidism which necessitated excision of three parathyroid glands, of which two showed adenomatous hyperplasia. In this patient transition from secondary to tertiary hyperparathyroidism probably occurred.

The term "tertiary hyperparathyroidism" was first suggested by Walter St. Goar (2) to describe patients who develop parathyroid adenomata causing hypercalcaemia on top of reactive or secondary parathyroid hyperplasia. In 1968 Davies et al. (3) established the syndrome on a firm foundation. In their first 200 cases of primary hyperparathyroidism proved at operation the authors found 12 patients with parathyroid adenomata which probably developed on the basis of long-standing hyperplasia due to malabsorption or chronic renal glomerular failure. A probable case of tertiary hyperparathyroidism is described.

CASE REPORT

A woman, aged 30 years. She was very much like her twin sister until the age of 14–15 years. At that time her weight was 68 kg and she was in good health. At the age of 13, menstruation began, but disappeared after 1 year and ever since she had amenorrhoea. During the following years she had a restricted calory intake and from the age of 18 years (1957) she suffered from vomiting and was several times treated in hospital. Her condition was regarded as anorexia nervosa.

In 1957 her weight was 40 kg. She had normal urine (specific gravity 1 030). Blood urea was 30 mg/100 ml. In 1959 the urine was still normal (specific gravity 1 018–1 024). Blood urea 33–38 mg 100 ml. Serum creatinine 1.6 mg 100 ml. Serum chlorides varied from 73 to 82 mEq/l. Serum potassium 3.0 mEq/l, serum calcium 12 8 mg/100 ml, and phosphorus 2.5 mg/100 ml. The alkali reserve was 85 mmoles. The ECG showed prolongation of the Q–T interval. She was severely disturbed emotionally.

She received treatment with solutions of sodium chloride intravenously and high caloric foods, and her weight rose to 46 kg. During the following years, however, her calories were restricted and she continued to vomit.

In 1963 she had anaemia (Hb 55 %), ESR 106 mm/h, proteinuria (acid urine, specific gravity 1 010–1 014). Blood urea 145 mg/100 ml, serum creatinine 0 8 mg/100 ml, serum calcium 10 7 mg 100 ml, serum phosphorus 4 8 mg 100 ml, and serum potassium 2 8 mEq l. Her vomiting continued unaltered during the following years.

On admission to our department in November 1968 she was extremely thin and pale with clinical signs of dehydration. She had papillary atrophy of the tongue, concave nails, dry hair and scanty axillary and pubic hair. There was red and tender swelling of some joints in the fingers and toes and cystic swelling of the bursa in the right cubital region. She had small haemorrhages in the skin on the arms and shoulders. B P 115 70 mm Hg.

Hb 7 4 g/100 ml, ESR 100 mm/h, red blood cell count 2 74 mill. mm^3, white blood cell count 6 800 mm^3, platelet count 216 000/mm^3. The urine contained protein (0 025 %), had a specific gravity of 1 011 and pH 6.4. Microscopy of the urine revealed 2 3 granular cells but no casts.

Blood urea 152 mg 100 ml, serum creatinine 6 3 mg/100 ml, serum sodium 134–153 mEq/l, serum potassium 2 8–4 9 mEq/l, serum chloride 75–89 mEq/l, serum calcium 5.3 mEq/l, serum phosphorus 7 5 mg 100 ml, serum alkaline phosphatase 44 U/l. The total protein in serum was 7 8 g 100 ml with normal electrophoretic pattern. Serum iron 30 µg 100 ml, TIBC 420 µg 100 ml. In capillary blood, pH was 7 45, pCO_2 46–70 mmHg, standard bicarbonate 30 mEq/l. Calcium excretion in the urine was 4 1–4 4 mEq/24 h. Only trace amounts of fluorine were demonstrated in the urine. The pentagastrin test showed a maximal output of 20 5 mEq/l. ECG showed prolongation of the Q–T interval 0 46 sec (normal 0 38 sec)

Radiological examination revealed deposition of cal-



Fig 1 X-ray of the hand showing deposition of calcium in the periarticular tissue of the swollen joints.

cium in the periarticular tissue of the swollen joint (Fig. 1), in the cartilagines of the larynx, and in the gluteal muscles. There was no renal deposition of calcium and no osteoporosis. The condition was regarded calcinosis interstitialis uni ersalis. Cystlike lesions were present in the metacarpals, which suggested hyperparathyroidism. Calcium salts were found in little nodule which w removed from the subcutaneous tissue.

The patient w studied by an ophthalmologist who found grey and white deposits in both corneae, hich implied deposition of calcium.

There normal excretion in the urine of 17-ketosteroids and 17-hydroxycorticosteroids.

The patient received high caloric foods, blood transfusions, electrolytes and amino acids, itamins and iron intravenously but she continued to vomit and her condition remained unchanged. Serum calcium rose to 6.6 mEq/l. No significant suppression of the hypercalcaemia was noted by the administration of prednisone 20 mg for 6 days.

On January 17 1969 the patient was operated on. Three of the parathyroid gland er found to be enlarged and were emoved. The fourth gland looked normal and w left behind. Histological examination showed adenomatous hyperplasia in two of the removed parathyroid glands. The tissue as normal in the third gland.

During the first 3 months after the operation s gained 5. kg in weight. The swelling of the joint as reduced. Radiological examination showed distinct egress of the calcium deposits. Serum ca.cium fell to 5.1 mEq/l, blood urea to 72 mg/100 ml, and serum creatinine to 4.2 mg/100 ml. Seven months after the operation her serum calcium was at lower level of normal range but her serum creatinine increased.

DISCUSSION

The patient had primarily anorexia nervosa with continuous vomiting which lasted for many years. Loss of the gastric juices over a long period may give rise to profound disturbances in the organism's acid–base equilibrium and electrolyte balance (1) and to both functional and morphological changes in the kidneys (4, 5).

For some years she had chronic renal failure and probably went through a phase of secondary hyperparathyroidism during which two of the glands became autonomous adenomata. They then produced the biochemical changes described as tertiary hyperparathyroidism. It seems unlikely that the patient suffered from primary hyperparathyroidism which was missed initially. Primary hyperparathyroidism with renal insufficiency would probably have shown a more rapid progression of the renal insufficiency during the last years. In 1959 the serum calcium was 1..8 mg/100 ml but the specific gravity in the urine 1.0?4. There was no evidence of excess vitamin D medication. She had not used diuretics or laxatives. An initial anorexia nervosa with continuous vomiting resulting in secondary renal insufficiency and tertiary hyperparathyroidism seems to be more likely.

After the operation the renal function temporarily improved, but on follow-up her renal failure seemed irreversible.

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PLATELET ADHESIVENESS IN CORONARY HEART DISEASE

Evaluation of the Plateletrich Plasma-ADP Method

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Abstract. A study of platelet adhesiveness using platelet rich plasma with ADP has been performed in different groups of men. Despite large day-to-day variations the method registered individual characteristics in platelet function. A seasonal variation and an increase in adhesiveness with age were found, but the method failed to show any difference between prospective and post-infarction group of the same age. Thus this variant for assessing platelet adhesiveness is not useful for detecting the coronary-prone patient.

There has for years been an intensive search for methods to disclose an increased tendency to thrombosis. The interest has mainly centered on various coagulation tests which might reveal "hypercoagulability".

The demonstration of decreased platelet adhesiveness to glass in bleeding disorders like thrombasthenia and von Willebrand's disease (4, 7, 10, 13) indicated that this parameter might reflect physiological platelet function. This recognition fostered the now widespread opinion that an increased adhesiveness to glass might reflect an increased tendency to thrombosis, a view that was already prepared for by the work of Wright (15) on venous thrombosis and platelet adhesion. Further, the decisive importance of platelet adhesion and aggregation in the initial stages of thrombus formation speaks in favour of this view. Platelet thrombus formation has its greatest clinical implication in the most important type of thrombosis, the arterial, and reliable methods for studying platelet function in clinical cases are therefore highly wanted.

After the introduction by Hellem (7) of a standardized glass bead method for measuring adhesion, great enthusiasm for this problem ensued and led to the development of many variants of the technique (8). The value of the different modifications in unveiling an increased tendency to thrombosis, however, has not been clearly established. Judging from the experience in von Willebrand's disease, where e.g. Salzman's method reflects a clear-cut decrease in adhesiveness whereas the original Hellem method does not, this is necessary. Moreover the existing studies have often been performed in the post thrombotic period, and findings might therefore have reflected only secondary changes.

The following is an attempt to evaluate the possible usefulness of the plateletrich plasma/ADP adhesiveness to glass test for the detection of the coronary-prone individual. The results, based on about 000 adhesiveness tests in CHD patients and in normals, were negative.

MATERIAL

The following groups were studied:

1. Forty soldiers, aged 20–22, mean 21.

2. Forty-three normal men, aged 40–50, mean 46, with no history of cardiovascular disease.

3. A cohort of prospective group for the evaluation of the possible prophylactic effect of an unsaturated fatty acid (linoleic acid) on coronary disease. The total material consisted of 16 600 men, aged 50–60. The details are described by Natvig et al. (11). The material was randomized in tw groups, one in linseed oil and one on sunflower oil, the code being know only to the oil distributor. The cohort used for the adhesiveness study consisted of 49 men from each group who volunteered to present themselves at the Institute for Thrombosis Research for drawing of fresh blood. The tests were made prior to the start of the experiment and every three months during the observation year. Since the statistical evaluation showed that there as no difference in the

adhesiveness between the linseed and the sunflower oil group, the two groups have been treated as one in the report.

4. A post-infarction material from Ullevaal Hospital, Oslo. This material has been described in detail elsewhere (3). The age ranged from 41–70, with mean of 57 Also this material as randomized in two groups, one on linseed oil and the other on corn oil. Again, since the statistical evaluation showed no difference between the groups concerning adhesiveness, they have been treated as one group in this report. In the beginning they ere assayed using 0.1 μg ADP/ml PRP but the figures mainly used in this report are those obtained by successive testing of 152 patients using 0 05 μg ADP/ml PRP

METHODS

Blood sampling This was always done between 8–9 a.m. Most of the participants had had light breakfast, mostly coffee and a sandwich. Preliminary investigations had show that this meal did not influence the results. The blood was drawn directly into 1/10 volume of 0.11 M sodium citrate placed in premarked siliconized glass tube. For practical reasons the citrate concentration was not adjusted unless the haematocrit was either above 50 or below 40.

Plateletrich plasma (PRP). Blood was spun at 1200 rpm (320 g) for 15 min at +4 in an International Refrigerated Centrifuge and the PRP transferred into siliconized glass tube by means of siliconized pasteur pipette

Platelet adhesiveness was estimated by modification f the Hellem method using plateletrich plasma and ADP (14) The measurements were performed at room temperature 1 hour after withdrawal of the blood. The same technician, having four years of experience with the method, performed all assays. The standard error of this method, using automatic counting, is 1.2% as calculated from the results of duplicates.

Counting of platelets Platelets were counted automatically using Celloscope 101 as described previously (14)

Adenosine diphosphate (ADP). Calcium salt, dihydrate from Sigma Chemical Co., Mo., USA. A stock solution containing 400 μg ADP/ml (nominal) in Tris buffer/saline was prepared for 1 year use and kept in closed ampoules at −70°C in exactly measured amounts to be diluted by given volume f buffered saline after thawing. On measurement according to Holmsen (9) the absolute amount was less than the nominal, and hat is said to be 0 05 μg/ml is actually 0.04 μg/ml.

Statistical evaluation has been performed by Dr K. Westlund and his colleagues at the Life Insurance Company Institute for Medical Statistics.

RESULTS

Relation between platelet count in PRP and adhesiveness

Since the platelet count in PRP influences adhesiveness (14) it was necessary to examine to what extent this variable affected the results. The regression line for the relation between adhesiveness and platelet number as calculated from the prospective material was $Y = 0.0412 x + 16.49$ From this it can be deduced that the adhesiveness figures given are only slightly influenced by variations in platelet number However the number of platelets in the PRP constitutes part of the adhesiveness parameter since the correlation coefficient calculated from the above material was +0.360.

Relation between haematocrit and adhesiveness

Since PRP and not whole blood was used in the present method, the only influence expected from the haematocrit would be on the relation citrate/calcium, i.e. the availability of calcium ions, which is of importance for ADP adhesion and aggregation (7). Since for practical reasons we allowed a variation of the haematocrit between 40 and 50 without adjusting the citrate concentration, we should expect some influence from a varying haematocrit on our results. Statistical calculations in two of the materials showed an expected negative small correlation, the correlation coefficients being −0 168 and −0.210, respectively Both were just significant at the 5% level. It can therefore be concluded that, although the haematocrit also constitutes part of the present adhesiveness parameter it has only a modest influence on the results.

The elation between the adhesiveness values with 0.05 and 0 1 µg ADP/ml PRP

In many persons simultaneous estimations of adhesiveness were performed, using both 0.05 and 0.1 μg/ml PRP Evaluation of these figures showed that the 0.1 adhesiveness could be predicted with high confidence by doubling of the 0 05 adhesiveness when the figures were below 30 for the latter Above this figure an increasing discrepancy in the predicted and actual values appeared. The ratio 0.1 ADP/0.05 decreased from . to 1 7 for the highest adhesiveness values.

Intra- and interindividual variations

Table I gives an example of the day-to-day variations in five volunteers tested at intervals of two (or three) days. An appreciable variability is evi-

dent, the variance being 56.6 with a mean adhesiveness of ∠7.0. The same variance was also found by using the data obtained in the prospective material with intervals of three months between the tests.

With such great intra-individual variations the question arises whether adhesiveness as measured by the present technique can pick up differences between normal persons of comparable age and sex. An analysis of variance of the results in the prospective material (Table II) showed a variance between persons of 532.2, the resulting F amounting to 9.40 which is highly significant. It is therefore clear that the measurements pick up some characteristic difference between

Table I. *Day-to-day variation in PRP/ADP adhesiveness*

The figures represent per cent adhesiveness as calculated from the differences in platelet count in PRP before and after passage of the column. Final concentration of ADP 0.05 μg/ml PRP

Subject no.	Date 1	2	3	4	5	6	7	8	9	10
1	22			25			10		12	
2		30		36		29		21		41
3	20		25		16		16		17	
4		25		28		15		30		25
5	37		35		39		42		53	

Table II. *Interindividual variance as compared to intraindividual variance*

	Degrees of freedom	Mean square	F[a]
Interindividual variance	96	532.20	
Intraindividual variance	341	56.64	9.40

[a] F variance ratio = variance between individuals divided by variance within individuals.

Table III. *Seasonal variation in adhesiveness and platelet number*

	Feb./Mar.	May/Jun.	Aug./Sep.	Nov./Dec.	Feb./Mar.
Adhesiveness,	33.5	41.0	31.0	37.6	35.8
Platelet number 10^{-3}	412	441	418	417	406

Table IV. *Relation of platelet number in PRP to age and coronary heart disease*

Subjects	Normals			Post-infarction
Age, mean (y.)	21	46	56	57
No.	40	47	459	152
Platelet no. in PRP 10^{-3}	381	431	414	396

Table V. *Platelet adhesiveness in different age groups of normals as compared with post-infarction group*

	Normals			Post infarction group
Age, y	21 (20–22)	46 (40–50)	56 (50–60)	57 (41–70)
No.	40	43	459[a]	152
Adhesiveness, %				
0.05 μg ADP/ml PRP	21 ± 8	22 ± 11	36 ± 12	7 ± 12
0.1 μg ADP/ml PRP	43 ± 8	46 ± 10	66 ± 10	66 ± 9

[a] Representing mean of 4.7 observations in 98 patients at three months intervals.

persons, but on the other hand the great variability within persons calls for large groups in order to obtain meaningful conclusions.

Seasonal variations

As the prospective material was tested at intervals of three months, a possible seasonal variation could be investigated. Table III shows the per cent adhesiveness as related to season, together with the comparable platelet numbers in the platelet rich plasma. The statistical calculation showed that the differences between May/June and February/March or August/September are significant even when the influence of the concomitant increase in platelet number is eliminated.

Relation between platelet count in PRP, age and coronary heart disease

Table IV shows the platelet number in PRP as related to age and coronary heart disease. The difference between young and middle-aged normals is significant ($p < 0.005$), whereas the difference between the prospective and the postinfarction group is not. It is therefore unlikely that the platelet number in PRP is of any use as

Table VI. *Relation between adhesiveness and age*

Born	Adhesiveness (0.05 μg/ml PRP)
Prospective material	
1905–1909	37.3
1910–1915	34 7
Post-infarction material	
1905–1909	38.2
1910–1915	35.9

parameter in coronary heart disease. The reason for the difference in platelet number in PRP from middle-aged men as compared to young is un known.

Relation of adhesiveness to age

The results in Table V indicate that adhesiveness is correlated with age: after 50 years there seems to be a rise in adhesiveness. The tendency to increase with age was further substantiated by dividing the post-infarction material and the prospec tive material in groups below and above the mean age as apparent from Table VI.

omparison of adhesiveness in a prospective and st-infarction group of same age

able V shows the result of assaying the adhesiveness in different age groups as compared with the post-infarction material From the last two columns it is seen that there was actually no difference in adhesiveness between the two groups. This conclusion is valid for both 0 05 and 0 1 μg ADP/ml PRP

Adhesiveness of patients with renewed cardiovas cular episodes in the post-infarction group

During the time we used 0.1 μg/ml PRP only to evaluate platelet adhesiveness, the mean of 274 tests in this group was 65 8 In this period 27 patients had renewed cardiovascular episodes, and their mean adhesiveness was 66.5 i.e. not significantly different from the mean of the group.

Of 152 patients evaluated with 0.05 μg ADP/ml PRP the mean adhesiveness was 37 .. Twelve of them had renewed cardiovascular episodes, and their mean adhesiveness was 38.8, which is not significantly different from the mean.

Adhesiveness in persons suffering cardiovascular attacks in the prospective material

In the 98 patients studied for one year at intervals of three months, the mean adhesiveness with 0.05 μg ADP/ml PRP was 36.5 As expected, there were not many deaths of cardiovascular disease in this group, and actually only two cases were encountered. Their mean adhesiveness was 19 6 and 26.3 respectively and was therefore appreciably lower than the mean for the total group.

DISCUSSION

The great day-to-day variability encountered with the present method for assessing platelet adhesion is in line with experience using other methods (2, 7). The reasons might be many methodological (14) as well as real. The latter might stem from variations in plasma or platelets, both being subjected to influence from a variety of stimuli, e.g. diet, stress, hormones, etc. (2). Regardless of the causes, the great variability calls for large sample populations and caution in drawing conclusions. The statistics showed that the interindividual variation was far greater than the intraindividual, i.e. the method registers individual charcateristics whatever their significance may be. However there was no difference in adhesion as measured by the PRP/ADP method between agematched groups of men with or without established cardiovascular disease. This contrasts with the majority of findings with modifications using whole blood (8). Differences in methodology may explain these discrepancies. Firstly separation of red cells by centrifugation for the preparation of PRP leads to loss of the most adhesive platelets (14). Secondly the kinetics of the ADP supply is different in the two methods. In the PRP method a definite amount of ADP is added at a given time, whereas in the whole blood methods ADP is made available successively from the red cells. The absolute amounts are also most certainly different in the two variants. Thirdly the presence of red cells per se might be of importance. Postoperative adhesiveness studies (12) have also shown that the whole blood and the PRP method give different results.

The study revealed that platelet adhesiveness increased with age, in conformity with earlier findings (5) using the whole blood method. Calculations show that neither the slightly lower

haematocrit nor the increased platelet number in PRP could account for the difference observed between the young and old men. Whether this is related to the increasing tendency to thrombosis with age is unknown.

A seasonal variation in adhesiveness has been observed previously (6), although in anticoagulated patients. The variations might be artificial because the results are influenced by many factors, but this is not likely for the following reasons. The technique was meticulously standardized, using the same stock of ADP frequently controlled biochemically and the same experienced technician performed all assays. The glass bead columns were produced successively and the known increase in adhesiveness with column age (1) could therefore hardly be the cause. There is therefore reason to conclude that the seasonal variation is real and adds to the other variables necessary to consider in adhesiveness studies.

Judging from the present investigation, the PRP/ADP method of measuring platelet adhesiveness is not useful for detecting proneness to thrombosis. This does not, however invalidate its use in pharmacological studies, where it has certain advantages over the whole blood methods.

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PITUITARY ABLATION IN DIABETICS WITH SEVERE RETINOPATHY

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Abstract. Pituitary ablation has been performed in 20 patients with diabetic retinopathy including 18 with proliferative retinopathy One postoperative death occurred. The remaining 19 patients have been observed for periods ranging from 22 to 131 months. Pituitary ablation had distinct effect on the background retinopathy the haemorrhages and red dots decreased in number the phlebopathy improved, and newly formed vessels in the retina and iris disappeared in few cases. However the effects of operation seem to be of transitory nature. After some years recurrence in the form of background or proliferative retinopathy and/or rubeosis of the iris was observed in some of the patients. The average decrease in vision was 11% in the group as whole, but only two patients were blind in both eyes at the last follow-up examination. Pituitary ablation seems to have inhibited the development of both retinopathy and iridic changes, but only long-term observations in controlled clinical trials can decide hether this peration is to have permanent place in the treatment of diabetic retinopathy

Since 1956 we have in Århus Kommunehospital performed pituitary ablation in diabetics with severe retinopathy threatening vision. A preliminary report of the results obtained in the first 14 patients was presented in 1962 (5). Most of these patients were hypophysectomized, using the transmaxillosphenoidal approach by the method of Hamberger et al. (2). The report showed that hypophysectomy seems to delay the development of severe diabetic retinopathy in certain patients.

This clinical impression corresponded fairly well with the results which at that time had been obtained elsewhere (11). However such an impression was not satisfactory and it proved necessary to perform a controlled clinical trial in order to determine whether pituitary ablation is of value in the therapy of proliferative diabetic retinopathy This new trial was started in 1962, and the results from the first five-year period were reported in 1967 (6). This experiment clearly showed that hypophysectomy inhibits the development of proliferative diabetic retinopathy

In addition to the above-mentioned 14 patients, another two patients had been hypophysectomized before the start of the controlled clinical trial. Since 1962 four patients who did not fulfil the requirements for inclusion in the controlled clinical trial had been hypophysectomized or subjected to some other form of pituitary ablation.

The following is a report of the course in the 20 patients who underwent operation in the period from 1956 to 1965

CLINICAL MATERIAL AND MODE OF OPERATION

General clinical data of the 20 patients are listed in Table I. The series consisted of 14 men and six women. All the patients but tw were below the age of 50 years. The verage age was 35.6 ± 2 4 years, and the average duration of diabetes as 21 0 ± 1_ years at the time of operation. Eleven of the patients had proteinuria before the operation; tw had slightly levated values of serum creatinine (cases 19 and 20, viz. 1 8 and 1 6 mg). None of the patients had cardiac symptoms.

Total hypophysectomy performed either by the transmaxillosphenoidal or the transcranial approach, as performed in 13 patients. In five cases transmaxillosphenoidal hypophysectomy as attempted, but the operation as incomplete in one, and had to be abandoned in four owing to profuse bleeding. Transcranial hypophysectomy as later performed in three of the patients, hile section of the hypophyseal stalk as carried out in two Section of the hypophyseal stalk as primarily performed in one patient, in another patient Yttrium 90 was implanted into the pituitary gland.

The patients were hospitalized every three to six months and, after the lapse of some years, only once y ar One patient died within the first month after the operation The average time of observation for the 19 surviving patients was 62 months, and 13 patients are under observation for more than four years.

Table I. *General clinical data*

No.	Sex	Age at operation (y)	Duration of diabetes at operation (y)	Proteinuria	Operation*	Died after operation (mo.)	Causes of death
1	♀	38	29	−	Cran.	61	Coronary occlusion
2	♀	34	26	−	Stalk		
3	♀	25	20	+	Sphen.	61	Pulmonary embolism, pyelo-nephritis, coronary sclerosis
4	♀	46	21	+	Sphen. + cran.	24	Shock of unknown cause
5	♂	22	17	−	Sphen. + stalk		
6	♂	58	24	−	Sphen.		
7	♂	27	22	−	Sphen.		
8	♂	39	15	−	Sphen. + cran.		
9	♂	32	24	+	Sphen.		
10	♀	44	15	+	Sphen.		
11	♂	26	20	+	Sphen.	28	Coronary occlusion
12	♂	44	35	−	Sphen. + cran.		
13	♂	48	8	−	Sphen.	51	Perforated ulcer of the stomach, coronary sclerosis
14	♂	40	23	+	Sphen.	58	Coronary occlusion, embolism of the internal carotid artery
15	♀	25	14	+	Sphen.	59	Hypoglycaemia
16	♂	26	21	−	Sphen.		
17	♂	53	23	+	Sphen.		
18	♂	31	24	+	Sphen.	38	Coronary occlusion, nephropathy
19	♂	33	20	+	Ytt. 90		
20	♂	21	18	+	Sphen. + stalk	0	Pulmonary embolism

Cran. = transcranial hypophysectomy. Sphen. = transmaxillosphenoidal hypophysectomy. Stalk = section of the pituitary stalk. Ytt. 90 = implantation of Yttrium 90 in the pituitary.

SUBSTITUTION THERAPY

The postoperative treatment and subsequent substitution therapy were carried out in accordance with the lines laid down in previous publications (5, 6). After the operation it was possible to reduce the dose of insulin by one half in most of the patients. This effect proved to be permanent. At the last check-up, the requirement of insulin averaged 59 % of the dose before the operation. Diabetes insipidus developed in all cases, and pitressin was therefore given during the first weeks or months. Immediately after the operation, treatment with cortisone or prednisone was instituted. Thyroid function—as measured by the PBI—decreased, and after a few months thyroid medication was started. Amenorrhoea developed in all women.

COMPLICATIONS AND DEATHS

The immediate complications in the first 14 patients have previously been reported (5). During attempted transmaxillosphenoidal hypophysectomy in case 4, the right internal carotid artery was damaged and had to be ligated. Transitory haemiparesis and ophthalmoplegia resulted. The patient was successfully hypophysectomized by the transcranial approach three months later. Orthostatic hypotension developed postoperatively in three patients. Transitory paresis of the facial nerve occurred in one, and of the oculomotor nerve in another. Meningitis developed in case 12 after the operation, but after treatment with antibiotics it soon disappeared. In case 11 transmaxillosphenoidal hypophysectomy led to injury of the chiasma and permanent visual-field defect ensued. In case 1 epilepsy developed a few years after the operation, which was performed by the transcranial route.

One of the last six patients who underwent operation died (case 20). Hypophysectomy had first been refused, but as the patient insisted, an unsuccessful attempt at transmaxillosphenoidal hypophysectomy was performed, followed by section of the pituitary stalk. The patient died eight days after the intervention from embolism of the right pulmonary artery.

No postoperative complications were observed in the other five patients.

In addition to the above-mentioned patient, who died shortly after the operation, a total of eight patients died 24 to 61 months after the operation. The causes of death are given in Table I. Half of the patients died from occlusion of the coronary arteries, one from hypoglycaemia, and another from shock of unknown cause.

Autopsy was performed in seven of the nine cases. In five the sella turcica was found empty or with sparse

Table II. *Ocular data*

			Visual acuity[a]		Proliferations before operation					
					Localisation					
No.	Duration of observation (mo.)	Eye	Before operation	At last examination	Disc	Retina	Disc and retina	Stage[c]	Course of proliferations	Stage at last check-up[c]
1	57	R	1.0	1.0		+		I	Improvement, disappearance of new vessels	I
		L	0.67	F			+ +	II	Severe progression, vitr. haem., conn. tissue	III
2	131	R	0.5	0.05			+ +	II	Severe progression, vitr. haem., vessels, conn. tissue, detachment of retina	III
		L	0.5	0.33			+ +	I	Severe progression, vitr. haem., vessels, conn. tissue	II
3	59	R	H	L			+ +	III	Severe progression, vitr. haem., vessels, conn. tissue, detachment of retina	III
		L	F	L			+ +	III	Severe progression, vitr. haem. vessels, conn. tissue	III
4	23	R	0.5	0.4			+ +	II	No change	II
		L	F	H			+ +	II	Severe progression, vitr. haem., conn. tissue	II
5	91	R	0.67	0.05			+	I	Slight progression, prepapil. vessels, atrophy of optic disc	I
		L	1.0	0.33			+	I	No change	I
6	63	R	0.25	0.33	+			II	Slight progression	III
		L	0.33	L	+			II	Severe progression, vitr. haem. conn. tissue, cataract	—
7	82	R	0.67	1.0			+	II	Improvement, disappearance of new vessels	II
		L	0.5	0.5		+		II	Improvement, disappearance of new vessels	II
8	83	R	L	−L				—	—	—
		L	0.4	0.33			+	I	Slight progression, gradual increase in prepapil. vessels and conn. tissue	III III
9	79	R	F	F			+ +	III	No change	III
		L	F	0.15			+ +	III	No change	III
10	79	R	0.1	F			+ +	III	Severe progression, detachment of retina	III
		L	0.67	1.0		+		II	No change	II
11	22	R	−L	−L				—	—	—
		L	0.4	0.5			+	I	Improvement, disappearance of vessels, but formation of thin conn. tissue	III
12	81	R	F	F		+		III	No change	III
		L	0.2	0.1		+ +		III	Slight progression, detachment of retina	III
13	47	R	0.4	0.33				0	No change	0
		L	0.33	0.33				0	Slight progression, vitr. haem. prepapil. vessels	I
14	57	R	0.5	0.4	+ +			I	No change	I
		L	0.5	0.4				0	No change	0
15	53	R	0.5	0.4			+ +	I	Slight progression, disappearance of vessels, but formation of prepapil. conn. tissue	III
		L	H	−L			+	III	Severe progression, vitr. haem., vessels, conn. tissue, haem. glaucoma	—
16	70	R	H	−L			+ −	II	Severe progression, vitr. haem., vessels, conn. tissue, haem. glaucoma	—
		L	1.0	H				II	Severe progression vitr. haem., conn. tissue	III

Table II (*continued*)

No.	Duration of observation (mo)	Eye	Visual acuity[a] Before operation	At last examination	Proliferations before operation Localisation[b] Disc	Retina	Disc and retina	Stage[c]	Course of proliferations	Stage at last check-up[c]
17	45	R	0.1	0.2				0	Slight progression, retinal new vessels	I
		L	0.1	0.2				0	N change	0
18	25	R	0.15	0 25				—	—	—
		L	0 25	0.33		+		II	Slight progression, vitr haem., conn. tissue	II
19	37	R	F	0.4			+ +	III	Improvement, disappearance of vitr haem.	III
		L	0 4	L			+ +	III	Severe progression, vitr haem conn. tissue, detachment of retina	III
20	0	R	0 67					0		
		L	0 4				+ +	I		

[a] F = counting of fingers. H = hand movements. L = l ght perception.
[b] + = proliferative retinopathy + + = severe proliferative retinopathy prominati g into the vitreous cavity
[c] 0 = no proliferations. I = naked vessels. II = connective tissue and dense vascular proliferation (active stage). III = dense connective tissue with contracture, decrease of vessel system (stage of regression). — = fundus could not be seen

remnants of the pituitary gland consisting f atrophic cells. In one case the pituitary stalk had been transformed into glial tissue with distinct vascularization, but there were no pituitary remnants. Necroses of the pituitary gland was observed in the patient who died one week after section f the pituitary stalk.

OPHTHALMOLOGICAL OBSERVATIONS

Visual acuity

The visual acuities of the 20 patients before the operation, and of the 19 surviving patients at the last follow-up examination appear from Table II

As may be seen, the patients suffered from severe retinopathy threatening vision, only 22 out of 40 eyes showing visual acuities of 0 33–1.0 at the first examination.

Aggravation was observed in some of the patients at the last examination, 22 to 131 months later but it should be noted that visual acuities of 0.33–1.0 were still found in 17 eyes. The impairment of vision appeared in most cases after the lapse of some years.

Before the operation two patients had greatly reduced vision of both eyes (visual acuity below 0.1). In one of these patients (case 9) visual acuity improved to 0.15 in one eye after the operation, whereas vision was unchanged in the other patient (case 3). Reduction of visual acuity from 1 0 to perception of hand movements occurred in case 16 in one eye (the other eye was blind). Thus at the last examination only two patients had a visual acuity below 0 1 in both eyes. Pre-operatively seven patients had visual acuity less than 0 1 in one eye, whereas this was the case in 12 patients at the last examination.

If vision is expressed in per cent in the form of visual efficiency (10) instead of visual acuity it is possible to assess an average for a group of patients by means of a single figure. When calculated in this way the average visual efficiency of the 19 surviving patients before the operation was 51 7% as compared with 40.8% at the last examination.

Ophthalmoscopic findings

Prior to pituitary ablation proliferative retinopathy was present in one or both eyes in 18 of the 20 patients. The two patients without proliferations (cases 13 and 17) were operated on because of severe haemorrhages and exudates in the macula.

The localisation of the proliferations on the optic disc and/or retina in each patient appears from Table II in which also a classification of the proliferations in three stages has been made:

I. Naked vessels.

II Vascular proliferations with connective tis-

sue formation, dominated by the vascular changes (active stage).

III. Dense connective tissue formation with a tendency to contracture and decrease in the vessel system (stage of regression).

Naked vessels alone were present in nine eyes (stage I). Vascular and connective tissue proliferations in stage II were observed in 12 eyes, whereas ten eyes had to be classified in stage III. In six eyes (four patients) no proliferations were found. The fundus could not be estimated in three eyes on account of haemorrhagic glaucoma (cases 8 and 11) or posttraumatic cataract with synechiae (case 18). Table II shows that in 22 eyes proliferations were observed both on the optic disc and in front of the retina, in most cases rather advanced (+ +).

The course of the proliferative retinopathy is also shown in Table II. During the observation period no change was noted in ten eyes. In four eyes with slight proliferative changes the new-formed vessels disappeared without simultaneous formation of dense connective tissue. The improvement set in after the operation and lasted for the rest of the observation time. A vitreous haemorrhage disappeared in one eye (case 19).

Slight progression of the proliferative retinopathy was seen in eight eyes, and severe progression in 12. In 13 eyes the aggravation was due to severe haemorrhages in or behind the vitreous followed by vascular and connective tissue formation and—in three eyes—also accompanied by detachment of the retina. In seven eyes no haemorrhages were observed in the vitreous cavity in connection with the aggravation of the proliferative retinopathy (In two of the eyes naked vessels appeared after some years, in three others connective tissue was formed, and in the last two steadily increasing detachment of the retina was noted).

It is remarkable that the progression of the proliferative retinopathy in most of the patients did not occur until after the lapse of several years (in 1_ eyes after more than three years, and in five after 2–3 years). Only in three eyes was aggravation noted within the first two years after the pituitary ablation.

At the last check-up examination proliferative changes were still absent in three eyes, while six eyes were in stage I, seven in stage II, and 16 in stage III. In six eyes the fundus could not be evaluated. During the period of observation evolution from stage I to II or III was seen in four eyes, and from stage II to III in six. In two eyes without proliferative retinopathy formation of new vessels occurred after the operation.

In addition to the proliferative changes, common diabetic retinopathy—sometimes called background retinopathy—was found in all patients before the operation. This retinopathy consists of red dots (micro-aneurysms), haemorrhages and phlebopathy. The phlebopathy was rather severe in several patients. In 14 eyes the arteries were sclerotic, thin and of varying calibre, in few cases sheathed or transformed into white bands. Furthermore, sparse exudates were observed in most of the patients.

A few months after the pituitary ablation a considerable improvement in the background retinopathy seemed to be evident in most of the patients. The phlebopathy decreased numerous haemorrhages and red dots as well as the oedema of the retina and a few exudates disappeared. The retina assumed a more dry appearance. This improvement of the background retinopathy was observed in 17 eyes of 12 patients. In two patients the retinopathy seemed unaltered, while changes in the background retinopathy could not be assessed in the rest of the patients, often on account of very extensive proliferations. In a few cases retinal haemorrhages recurred after some years, but it was the general impression that these haemorrhages were less pronounced than those present before the operation. The improvement of the phlebopathy seemed to be permanent, but in a few cases the arterial changes aggravated with sheathing and obliteration.

Rubeosis of the iris and glaucoma

After the operation rubeosis of the iris disappeared in three patients. Two of these patients had haemorrhagic glaucoma in the contralateral eye, and the third patient bilateral rubeosis of the iris. In all three patients the rubeosis disappeared in the non-glaucomatous eyes and decreased in the eyes with glaucoma after pituitary ablation. None of the other 17 patients had rubeosis of the iris before the operation.

At the last check-up examination nine patients had rubeosis of the iris in 1 eyes, including four with haemorrhagic glaucoma. In two of the patients the glaucoma had been present pre-opera-

tively as mentioned above in the two others haemorrhagic glaucoma appeared $1\,^1/_2$ and $3\,^1/_2$ years after the operation. In the remaining five patients (eight eyes) slight rubeosis of the iris appeared 3–6 years after the operation.

Simple glaucoma was not found in any of the patients. The intra-ocular pressures were low in most cases. At the last examination the intra-ocular pressures were measured by an applanation tonometer in 16 of the patients, and the average pressure in the eyes without haemorrhagic glaucoma was 12.5 ± 0.7 mmHg.

DISCUSSION

Hypophysectomy or some other form of pituitary ablation exerts an inhibitory effect on the development of severe diabetic retinopathy. After the operation a pronounced improvement of the background retinopathy is observed, with haemorrhages and red dots (micro-aneurysms) decreasing in number or even disappearing. A marked regression of the phlebopathy occurred, and in a few cases disappearance of the newly formed vessels was noted.

This impression from the first operations has been confirmed by the observations of other authors (3 4 7 8) as well as by our controlled clinical trial (6). Furthermore, decrease or disappearance of rubeosis of the iris has been observed in a few patients (1 5 9).

The present observations, however, seem to indicate that the effect of the operation is of a transitory nature. After some years recurrence is seen in several of the patients in the form of haemorrhages in the retina and in the vitreous cavity as well as development of rubeosis of the iris. The haemorrhages and the rubeosis of the iris, however, seem to be less pronounced as compared with the pre-operative lesions. Likewise the phlebopathy did not seem to assume the severe character which was seen before the operation. Still it is difficult to speak with certainty about such lesions in a material like the present one, which is based on ophthalmoscopy alone and in which the pathological picture is so complex.

Long-term observation in series of controlled clinical trials will prove whether the advantage obtained bears a reasonable relation to the risk inevitably involved in pituitary ablation and the substitution therapy which is necessary after the operation.

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PLASMA RENIN ACTIVITY IN CHRONIC NEPHROPATHY

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Abstract. Plasma renin activity (PRA) has been measured in 14 patients with chronic nephropathy maintained on hemodialysis. Most of the patients were hypertensive at the time of measurement, and mean PRA was significantly elevated. No correlation between PRA, mean blood pressure and dialytic controllability of hypertension could be established. A in normal subjects and in hypertensives of varying etiology there appeared, however to be an inverse correlation between PRA and plasma sodium concentration.

In patients with terminal kidney disease, maintained on hemodialysis, the kidneys are often contracted to a degree not seen before the introduction of hemodialysis. In such extensively contracted kidneys Faarup et al. (5) have demonstrated heterotopic renin formation in the intra renal arteries and greater arterioles, whereas renin is exclusively present in the juxtaglomerular apparatus of normal kidneys (9) In the material of Faarup et al. most of the patients had hypertension, though at the time of bilateral nephrec tomy the pressure was normalized or reduced after a period of hemodialysis.

The demonstration of heterotopic renin formation in the contracted kidney has induced this attempt to measure plasma renin activity in patients with terminal renal failure, maintained on hemodialysis.

MATERIAL AND METHODS

The material consists of 14 patients. Details are given in Table I. The patients had been maintained on hemodialysis for periods varying from one to six months. The patients ere dialysed for twelve to fourteen hours twice week.

Plasma renin activity (PRA) was measured in Boucher et al. (4), modified by Nielsen and Møller (7). The coefficient of variation of the method is $\pm 12\%$. Normal supine PRA (measured 8–10 m.) is 14 ng angiotensin/10 ml plasma 4 hours incubation. Range 0–36 ng (8).

Plasma sodium concentration as measured by flame photometry using lithium as internal standard, confidence limits 95 = ± 2 mEq l.

Mean blood pressure calculated as diastolic blood pressure plus 40% of the pulse amplitude.

1. Supine PRA before nephrectomy PRA as meas ured concomitant with plasma sodium concentration on 15 ml blood drawn from the arterial end f the arteriovenous fistu of the 14 patients The patients had been recumbent for at least one hour before the blood as drawn, always 8–10 m.

2. Supine PRA after nephrectomy PRA as measured in ten patients seven days after nephrectomy Procedure as described above.

3 PRA was measured immediately before and after hemodialysis in four patients. The duration of the dialysis was between 12 and 14 hours. The dialyses ere carried out all around the clock. The weight changes ere between +100 g and −2 000 g. The changes in plasma sodium concentration did not xceed 4 mEq l. In no patient was significant change in PRA found. Hence the blood samples for measurements of PRA ere obtained disregarding time and dialysis.

RESULTS

1 *Supine PRA before n phrectomy*

The results are given in T ble I. Mean PRA 44 ng angiotensin/10 ml plasma 4 hours incubation ± 8 (S.E.M.) As estimated by Student *t*-test this mean value is significantly higher than nor mal ($p < 0.001$). Fig. 1 demonstrates the lack of correlation between simultaneous determinations of PRA and mean blood pressure. Fig. 2 demon strates that PRA in patients becoming normotensive during dialysis treatment did not differ from PRA in patients maintaining elevated blood pressure. Fig. 3 demonstrates the correlation between plasma sodium concentration and plasma renin activity in normal subjects and patients with different types of hypertension, determined in our laboratory The corresponding values for the pa tients of the present material are also given in

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PLASMA RENIN ACTIVITY AFTER ALLOGENIC KIDNEY TRANSPLANTATION IN MAN

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Abstract In ten patients who had received an allogenic kidney graft, plasma renin activity (PRA) and creatinine clearance have been followed by consecutive measurements in the first few months after transplantation, especially during the first month. PRA attained maximum value within the first days after transplantation, and then stabilized on lower value. With few exceptions PRA remained within normal range. No correlation was observed between PRA and fluctuations in creatinine clearance. None of the patients showed clearcut rejection crisis, and when slight signs of rejection appeared, no increase in PRA was observed. In five of the ten patients PRA was measured in supine and standing position, about one month after transplantation. There was a significant PRA increase, but the increase was less pronounced than in normal subjects.

Damage to the small vessels in the kidney is an early event in rejection of the allotransplanted kidney (8, 11 19). Correspondingly the creatinine clearance decreases at rejection (1 7 13 17). Thus the condition has features in common with experimental renal artery stenosis. Plasma renin activity (PRA) is frequently elevated in patients with this disorder (18) Lundgren et al. (13) have reported a regular increase in PRA in rejection crisis in man after allogenic kidney transplantation.

Thus, it has been found of interest to follow PRA and creatinine clearance in group of human kidney allotransplant recipients. No clear cut rejection crisis was observed, but the creatinine clearance varied considerably in the material.

MATERIAL AND METHODS

The material consists of ten patients, six males and four females, aged 21 to 50 (Table I). All had been maintained on hemodialysis for several months before transplantation. Nephrectomy was performed on an average eight weeks before transplantation, except in case 6 in whom nephrectomy was performed in the same session as transplantation, and case 88, who was nephrectomized four years before transplantation. The kidney diseases leading to nephropathy were chronic glomerulonephritis chronic pyelonephritis, polycystic kidney (case 61 and malignant hypertensive nephrosclerosis

All patients were normotensive at the time of transplantation, except patient 65 who had blood pressure of 180/120. During the period of investigation the patients received prednisone, 15–40 mg day and azathioprinum, 100–150 mg/day All patients er on liberal salt intake.

PRA was measured as Boucher et al. (4) as modified by Nielsen and Møller (14). CV on double analysis is ±12%. Mean PRA in normal supine subjects in samples obtained from 8 to 10 a.m. is 14 ng/10 ml plasma 4 hours incubation, ranging from 0–36 ng (16).

Colloid osmotic pressure (COP) as measured in an electronic osmometer for quick, direct measurement on small samples, described by Hansen (10). Results are given in cm H_2O The 95% confidence limits are _0 5 mmHg.

N and K concentrations in plasma and urine ere measured by flame photometry Creatinine concentration in serum and urine was measured with an autoanalyser

Supine PRA was measured on 15 ml peripheral venous blood. The patients had been recumbent for one hour The samples were obtained between 8 and 10 a.m except in the first 24 hours after transplantation, when they were obtained at any time of the day In all ten patients the first sample was obtained 1 to 3 hours after revascularization f the grafted kidney and was followed by measurements at intervals as indicated in Fig. 1 Six to seven PRA measurements are performed on each patient, mostly in the first month (Fig. 1). In six patients two to four PRA measurements were performed in the first 24 hours after transplantation The exact intervals are indicated in Fig. 1.

The following signs are taken as indications of incipient rejection: tenderness of the graft, increase in body temperature, increase in blood pressure, increase in body weight, decrease in creatinine clearance and diuresis, decrease in the urinary excretion of sodium, and occurrence of proteinuria or increase in present proteinuria (20).

Table I. *Age and sex of investigated patients. Postural experiments,* +

Case no.	Sex	Age	Postural experiment
55	♂	35	+
59	♂	25	+
61	♂	41	+
64	♂	44	−
65	♂	50	−
73	♀	34	+
78	♀	40	−
83	♂	44	−
88	♀	22	−
89	♀	21	+

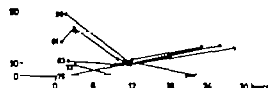


Fig. 1. Plasma renin activity in recumbent position (PRA) in the first 30 hours after transplantation. Time of revascularisation indicated by zero. Case numbers indicated in the figure.

Fig. 2. Plasma renin activity in recumbent position (PRA) and creatinine clearance (ml/min) after transplantation. Case numbers indicated in the figure.

Postural experiments were performed on six patients approximately one month after transplantation according to the following procedure. After the patients had been in a recumbent position for one hour PRA and COP ere measured on the same sample obtained without stasis. The patients assumed standing position, and after 20 and/or 30 min the measurements were repeated. In normal subjects PRA and COP have attained plateau values (15). The experiments were performed between 8 and 10 m.

In order to establish the influence of prednisone treatment on the postural renin response, postural experiments ere performed on three patients under treatment with prednisone 15–45 mg/day. This dose corresponds to the dose given to our transplantated patients. The controls had bronchial asthma and did not exhibit signs of cardiovascular or renal disease. It appears from Fig. 3 that the responses were normal. It has been considered unlikely that aldosteronism influences renin response.

RESULTS

Supine PRA after Transplantation

PRA in the first 24 hours afte transplantation (Fig. 1)

Immediately after revascularization, PRA was in the normal range in five patients. It was slightly increased in one (case 89 PRA 48 ng) despite a completely uneventful course after transplantation. There were no common trends in the PRA changes in the first 24 hours after transplantation, within the normal range increases as well as decreases were seen. Immediately after transplantation case 83 developed signs of rejection, as indicated by drop in creatinine clearance for four days, but there was no increase in PRA, neither at this time nor later on when the kidney function was normalized.

PRA in the first months afte transplantation (Fig. 2)

In cases 55 59 61 65 88 and 89 an increase in PRA is seen immediately or in the first days after transplantation. The PRA then stabilized on a lower value, or became unmeasurable, and increased somewhat later on. No PRA value was outside normal range, except the one mentioned above (case 89). In the above-mentioned patients, creatinine clearance quickly became satisfactory and in no case was more than one of the above-mentioned criteria of rejection observed. Blood pressure increases to hypertensive levels (diastolic blood pressure greater than 100 mmHg) were observed in cases 59 and 88 These increases in blood pressure were concomitant with PRA increases, but the PRA increases in cases 55 and 61 were not followed by blood pressure increases or other signs of rejection. In case 89 tenderness of the graft was present at a time when PRA was falling from its initial elevated value.

Four patients demonstrated a course deviating from the one outlined above (cases 64 73 78 and 83). Cases 64 and 78 showed greatly oscillating PRA values. In case 64 PRA was increased immediately after transplantation (45 ng). There were no signs of rejection, but creatinine clearance varied considerably There was no parallel ariation in PRA and creatinine clearance The patient was persistently normotensive. Case 78 showed positive signs of rejection, decrease in creatinine clearance and decreased excretion of sodium in the urine. At this time PRA was of the same magnitude as before the rejection crisis, and the value also corresponded well with PRA when the rejection crisis was overcome by a temporary increase in prednisone dosage. Case 73 was characterized by consistently low PRA, while the creatinine clearance was satisfactorily high. At a moment when PRA was unmeasurably low there were signs of rejection in the shape of graft tenderness. Case 83 had from the time of operation greatly diminished kidney function, interpreted as tubular damage to the graft. This patient showed increasing PRA in the first days after transplantation, yet PRA never exceeded normal range. PRA one month after transplantation, when renal function had become normal, was of a magnitude similar to that measured at the time of initial kidney damage.

Postural Experiments

Fig. 3 demonstrated the relation between increases in COP and PRA from supine to standing position. Correlation between ΔCOP and ΔPRA is linear -0.68 $p < 0.05$ The equation of the correlation line is $y = 2.25x - 8.6$

In Fig. 3 is indicated the corresponding regression line for normal subjects (16). It is readily seen that the values of the transplanted patients are significantly lower than normal.

DISCUSSION

Increase in PRA by rejection of kidney transplants is demonstrated by Abbrect et al. (1) in dog experiments and by Lundgren et al. (13) in man.

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THE EFFECT OF IRON SUPPLEMENTATION ON THE PHYSICAL WORK CAPACITY IN THE ELDERLY

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Abstract The effect of high oral iron supplementation (120 mg day) on the physical work capacity has been studied in group of apparently healthy men and women of ages 58–71 years, and this effect was correlated with the initial values of and changes in some variables constituting usual clinical estimates of the state of iron nutrition. The increase in physical work capacity was about 4% higher in men and about 12% higher in women in the group supplemented with iron than in the control (placebo) group. In the control group there as positive correlation between increase in physical work capacity and initial alue for stainable iron in the bone marrow and negative correlation between increase in physical ork capacity and decrease in the value for stainable iron in the bone marrow It is pointed out that these findings should not be expected in the iron-supplemented group, here ariations in the depot iron will be of less influence. In the iron-supplemented group the total iron binding capacity decreased significantly while the remaining variables constituting estimates of the state of iron nutrition showed no significant changes. The increase in physical ork capacity in the iron-supplemented group did not appear to be correlated to the initial value either for haemoglobin concentration or for other variables estimating the state of iron nutrition. It is concluded that in apparently healthy people the increase in physical work capacity during moderate training is related to the availability of iron.

Lower values for haemoglobin concentration (Hb) have been shown in elderly men compared with young and middle-aged men by several workers (15 16 18). Josephson and Dahlberg (18), like several others, found lower values in adult women than in adult men, but in women the values did not decrease with increasing age.

McDonough et al. (19) reported gradually increasing values in women of ages 15–54 years, and unchanged or slightly decreasing values in women aged 55–74 years. Pirrie (20) observed gradually decreasing serum iron (S-Fe) values in persons aged 30–80 years. Similar results have been obtained in several investigations on comparison between 70-year-old and 20–30-year-old men and women (16, 18, 21). The total iron binding capacity (TIBC) also seems to be correlated to age, however since Reckenberger (21) found the same changes with age for total iron binding capacity as for S-Fe.

The daily iron intake has been found to be strongly correlated to the daily total caloric intake (6) The caloric intake of the elderly is, as a rule, lower than that of young, active persons In a dietary study of a sample of pensioners over 67 years of age, carried out in the years 1959–1965 calculations showed relatively low mean iron intake, viz. 8 mg in women and 10 mg in men, at a mean intake of 1 540 and 1 905 calories, respectively (1).

There are no accurate estimates of the dietary iron need. Calculations of the degree of absorption, the net requirement and losses are rather unreliable. It can be assumed that diseases giving rise to reduced iron absorption, such as trophic gastritis, are more frequent at higher ages. On the other hand the dietary iron requirement of the elderly may be lower than that of younger persons, owing to a release of iron caused by muscle involution and reduction of the blood volume (13).

The diagnosis of iron deficiency is based, as a rule, on indirect method of measurement. It seems to be generally presumed that the body iron is, in principle, distributed between the different iron compounds according to laws of equilibrium. A deficiency found in one or more of these compounds thus reflects similar deficiency in other iron compounds investigated. Thus, in past population studies, one or a combination of different compounds such as haemin-bound

Table I *Physical characteristics of the material*

Sex	Group	No.	Age, y Mean	Age, y Range	Height, cm Mean	Height, cm S.D.	Height, cm Range	Weight, kg Mean	Weight, kg S.D	Weight, kg Range
♂	Iron group	13	64.8	58–71	175.6	8.3	164–184	74.8	9.7	58.5–95.0
	Placebo group	12	64.4	60–71	174.2	6.2	166–182	75.5	5.6	66.0–85.0
♀	Iron group	10	64.7	60–70	162.3	7.0	150–178	60.1	7.3	52.0–68.5
	Placebo group	10	63.0	59–66	163.6	5.4	154–168	66.8	8.8	46.5–78.5

Iron, serum iron, total iron binding capacity or measures of non-haemin iron in the marrow have been used as expressions of the so called depot iron The wide variation in the individual variables, even in apparently healthy persons, combined with the fact that they form part of kinetic systems which are influenced by many other factors than the availability of iron, means that definitions of the term iron deficiency based on these variables must be taken with considerable caution.

It has been claimed—although not generally accepted—that iron deficiency can give rise to un- general symptoms, even when anaemia in accepted sense is not present. Some investigators have considered that these symptoms might be due to reduced activity in different iron-containing enzyme systems (2, 3 4 5 17) However Wood and Elwood (25) found no significant correlations between the degree of some such general symptoms and Hb. Elwood, giving iron and a placebo did not observe any significant difference between the treated and control groups with regard to the influence upon these symptoms. On the other hand the difference in effect on Hb was significant (7). Swedish investigations have given similar results (1.)

There is no generally accepted definition of iron deficiency based on Hb, haematocrit, S-Fe, TIBC or estimated iron content of the bone marrow As has been pointed out by Garby et al. (11) it might even be considered that a criterion based on the individual person's feeling of well being and social functional capacity would be theoretically the most relevant one. The physical work capacity might be a suitable measure in this connection.

The purpose of the present investigation was to study whether

1 the physical work capacity in apparently healthy persons of ages 60–70 years is increased by an additional intake of iron

2. an additional intake of iron in persons of these ages causes a change in Hb or in the conventional clinical measures of the state of iron nutrition such as S-Fe, TIBC or non-haemin iron in the bone marrow

3 there is any correlation between changes in physical work capacity produced by an additional intake of iron, and changes in total haemoglobin (THb), Hb, S-Fe, TIBC and non-haemin iron in the bone marrow

4 there is any correlation between changes in physical work capacity and in iron stores in apparently healthy persons of ages 60–70 years who are repeatedly examined by submaximal physical work tests.

MATERIAL

The series comprised 45 (20 ♀ 25 ♂) voluntary clinically healthy persons of ages 57 71 years. They were chosen among persons followed up in 1966 subsequent to a General Health Survey in 1961 on a sample of the urban population of Uppsala. The original series has been presented previously (9) Only those who were included in the analysis of data from the 1966 study were asked to take part in the present investigation.

Of 52 women and 47 men who were included in the 1966 study 40 and 35 respectively were asked to participate in this investigation. For the following reasons the remaining 12 women and 12 men were not asked. two of them had died, ... were considered incapable of collaborating in an investigation of this kind, and in 20 persons there was known or suspected disease which might possibly have influenced one or more of the variables to be studied. Three persons did not answer the inquiry about participation, 18 refused, and a further six

Table II. *Mean values, standard deviations and ranges for haemoglobin concentration (Hb, g%), total haemoglobin (THb, g) and indices of physical work capacity (W_{170} and $W_{max\ perf}$, kpm/min) compared with the corresponding data for the original series*

Sex	Group	Hb g%			THb g			W_{170}, kpm/min			$W_{max\ perf}$, kpm/min		
		Mean	S.D	Range	Mean	S.D	Range	Mean	S.D	Range	Mean	S.D	Range
♂	Iron group	13.2	0.81	12.0–14.6	667	98.7	560–939	653	130.6	431–877	845	154.4	650–1 200
	Placebo group	13.4	0.69	12.3–14.8	643	54.4	539–677	585	116.2	393–822	825	120.9	600–1 000
	Original series	13.3	0.85	11.6–15.4	697	91.0	489–906	589	134.1	360–880	833	161.1	400–1 200
♀	Iron group	12.7	0.71	11.4–13.7	468	70.5	374–587	320	113.5	86–499	463	83.6	383–600
	Placebo group	12.3	0.37	11.8–13.1	505	95.6	339–655	366	112.0	160–543	552	116.6	400–800
	Original series	12.6	0.70	10.8–13.7	543	75.9	353–762	320	130.4	80–550	499	109.7	317–800

were prevented from taking part. In three subjects the examinations were not completed, the reasons being family matters, prolonged bronchopneumonia and gastrointestinal side-effects of the trial preparation, respectively. After these exclusions the series thus comprised 45 subjects (20 women and 25 men) for whom haematological data could be analysed. One subject refused to take part in the physical work tests, and in one the work tests could not be fulfilled as the subject fainted during the first test. From the analysis of the physical work capacity one woman and three men were excluded. In two of these cases the reason was unwillingness to perform a further exercise test; in one case the test, which was carried out on a one-leg bicycle, was technically unsatisfactory and in the fourth case another doctor had prescribed a sympathomimetic drug during the investigation period.

In all subjects a detailed history was taken and a clinical examination performed, including physical examination, urine tests for blood, protein, sugar and acetone bodies, serum creatinine and a Hematest® faecal examination. Two persons showed a positive reaction to the Hematest; a further test was negative, and rectoscopy and roentgenological examination of the stomach and colon were also performed. In one person colonic diverticulae and deformation of the duodenal bulb were observed.

Table I presents the physical characteristics of the material. The data agree in all essential respects with the corresponding data for the original series (10).

Table II presents the mean values, standard deviations and ranges for some of the variables studied, as well as the corresponding values for the original series. For Hb and physical work capacity the two series are in good agreement, while the mean value for THb was somewhat higher in the original series.

METHODS

The investigation was performed as a single-blind study with the iron-treated subjects and the controls grouped pairwise, each sex separately. An even distribution of treated and control persons was obtained throughout the period of the investigation, thus any seasonal variations could be expected to affect all groups similarly. The participants were requested not to take any drugs during the investigation period and to contact the author in the case of any intercurrent illness.

The iron-supplemented subjects (hereafter referred to as the iron group) were given ferrous fumarate orally in a dose of 60 mg (Fe^{++}) twice daily between meals, and the controls (placebo group) were given a specially prepared placebo. The duration of treatment was three months. The tablets were issued in packages of 60, giving the date by which each package should be consumed. By repeated telephone inquiries a check was made that the tablets were being taken as prescribed. At the end of the investigation the numbers of tablets not consumed were counted. In this way a check was made that the instructions were being followed, and information was also obtained on any intercurrent illnesses or conditions which might have affected the investigation. All participants, except four, took all tablets, and of these four none had more than 15 tablets left. One of the participants (a control subject) had to discontinue the treatment after one month because of gastrointestinal disorders. Otherwise there were no symptoms of intolerance.

All subjects were examined in the same way on all occasions with respect to time of day and the mutual timing of the different stages of the investigation. They came to the laboratory in the morning, after a night's fasting, and the programme was started with determination of total circulating haemoglobin (THb) and blood sampling in sitting position. A careful case history was taken and a physical examination performed. The next stage was a physical work test, followed by bone marrow puncture. With the exception of the work test and the marrow puncture, duplicate determinations were always aimed at. Also the physical work test was performed in duplicate by 21 of the 45 participants (11 men and 10 women), but all results of the present study are derived from the work test of the first day.

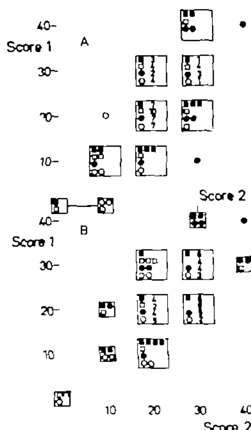


Fig. 1. Scores for non-haemin iron in bone marrow obtained by the two examiners (A and B) plotted against each other. The figures next to the symbols indicate the number of symbols of the same kind within the square. All symbols within the same square should coincide with the point of intersection of the diagonals of the square. ■ ● iron group, men and women, respectively. □ ○ placebo group, men and women, respectively.

Haemoglobin concentration

Hb was determined spectrophotometrically as cyanmethaemoglobin, using Aculute solution (Ortho Pharmaceutical Corp. New Jersey USA). The samples were taken as capillary blood after the subject had been sitting for at least 30 min. Sampling was performed on two consecutive days, and on each occasion a duplicate analysis was made on samples from the same finger. The mean value for all determinations was calculated. The error of the method ($\delta = \pm\sqrt{\Sigma d^2/2n}$) for Hb, calculated on 50 duplicate determinations for men and 42 for women, was ± 0.42 g% for the former and ± 0.43 g% for the latter corresponding to 3.16% and 3.46% of the mean value for the respective sexes.

Serum iron and total iron binding capacity

S-Fe and TIBC were determined at the Department of Clinical Chemistry, University Hospital, Uppsala, using an automated equipment with a Perkin Elmer Atomic Absorption Spectrophotometer 303. Corrections were made for haemoglobin in the serum. TIBC was estimated as total iron after addition of ferric chloride and absorption of the non-bound iron by magnesium carbonate. Duplicate determinations were performed on serum taken from two blood samples on each sampling occasion, and the mean value was calculated. All analyses of S-Fe and TIBC were performed on one occasion and by the same technician.

The error of the method ($\delta = \pm\sqrt{\Sigma d^2/2n}$) for S-Fe for the men was ± 7.3 μg% or 6.6% of the mean value, and for the women ± 5.6 μg% or 5.8% of the mean value. For TIBC the corresponding figures were ± 2.7 μg% or 0.8% for the men, and ± 2.9 μg% or 0.9% for the women.

Non-haemin iron in the bone marrow

The marrow puncture was performed in the sternal body or manubrium, using a relatively coarse puncture needle. About 2–3 ml of a mixture of blood and marrow were usually aspirated. Any blood present in the mixture was absorbed by gauze compresses. At one end of the object glass the bone marrow was allowed to dry in its existing state, and at the other end the marrow was spread as a relatively thick layer. Several slides were prepared. The preparation which was assessed to contain the largest number of marrow particles of suitable thickness was used for iron staining. In cases when the quality of the stained preparation was considered doubtful, other preparations were available for further staining. The staining was performed according to the method of Hansen and Weinfeld (14) without counter-staining. The preparations were stored, and their evaluation was deferred until the whole series was completed. Before evaluation the preparations were numbered according to a code system which made it impossible for them to be identified by the examiners. Two examiners who had had considerable practice in making evaluations on a uniform basis made two evaluations each independently on the same preparation, with an interval of more than one week. The preparations were studied in a microscope with high magnification (× 800). The amount of reticular iron was estimated semiquantitatively according to a five-grade scale (0, +, ++, +++, ++++), where

0, no haemosiderin granules found in the whole preparation;

+, only a few granules found in the whole preparation;

++, small granules found in every second or every third immersion field;

+++, large granules found in every immersion field; and

++++, massive haemosiderin deposition with aggregations and large granules.

In the subsequent calculations every + was given 10 points, and the mean value from all four evaluations was calculated. The gradation was based in principle on intracellularly located granules, but owing to the difficulty in deciding what was intracellular and what was extracellular this principle could not be strictly upheld. Only well demarcated, typical granules were accepted. Outflowing scales of stained material were regarded as

impurities. In any doubtful cases, new preparations are stained.

In Fig. 1 the values obtained at the two evaluations of each preparation by each examiner separately are plotted against each other. It can be seen that the reproducibility for the two examiners was essentially the same. For 48% of the preparations no difference was found between the two evaluations, for 50% the difference was 10 units, and for 2% it was 20 units.

In Fig. the mean values obtained by one examiner are plotted against those of the other. There was very good agreement between these values.

Total haemoglobin (THb)

This was determined by the alveolar CO method (24). Duplicate determinations were performed on all subjects. The errors of the method ($\delta = \pm\sqrt{\Sigma d^2/2n}$), calculated on 49 duplicate determinations in the men and on 41 in the women, were 30.9 g and 19.2 g, respectively corresponding to 4.6% and 3.9% of the mean value for the respective series (13 of 25 men and 3 of 20 women are smokers).

Blood volume

The total blood volume (TBV) was calculated as the ratio between THb and Hb, with correction for the difference between the body haematocrit and the haematocrit in capillary finger blood, according to the equation

$$TBV\ (l) = \frac{THb\ (g)}{9.1 \cdot Hb\ (g\ \%)} \quad (II)$$

The error of the method ($\delta = \pm\sqrt{\Sigma d^2/2n}$) was ± 0.33 for the men and ± 0.26 for the women, calculated on 46 and 42 duplicate determinations, respectively and corresponding to 5.9 and 5.9% of the mean value for the respective series.

Lactate concentration (lact, mEq/l)

Two consecutive capillary blood samples were taken, commencing at rule 15 sec before termination of the exercise test, for determination of the lactic acid concentration. The samples were analysed according to Ström modification (24) of the Barker and Summerson spectrophotometric method. The highest of the values from these two samples ($lact_{max}$) served as the basis for calculation of one of the indices expressing the physical work capacity (W_{lact}).

Physical work capacity

The physical work test as performed on an electrically braked bicycle ergometer according to method described previously (10). The same bicycle ergometer and ECG apparatus are used for all work tests and were calibrated at regular intervals. The paper speed as checked before and after each exercise test.

The values for physical ork capacity (W_{130}, W_{150} and W_{170}) were obtained by numerical extrapolation or interpolation. Extrapolation was not performed for more than 20 beats/min (10). The physical ork capacity is also expressed as the highest attained work intensity ($W_{max\ perf}$). (For definition see (23) and for nomenclature (22).)

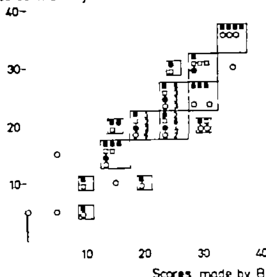


Fig. 2. Mean values for non-haemin iron in bone marrow obtained by the two examiners (A and B), plotted against each other. The figures next to the symbols indicate the number of symbols f the same kind within the square. All symbols within the same square should coincide with the point of intersection f the diagonals f the square. ■ ● Iron group, men and omen, respectively. □ ○ placebo group men and women, respectively.

The relationship $W_{max\ perf}$ log $lact_{max}$ as used for calculating the ork load ($= W_{lact}$) at an arbitrary blood lactate level (5 mEq/l) (30).

In the practical interpretation of the results the expression $\Delta W_{max\ perf}$ was introduced. (Definition, see Discussion of method, and also Table IV.)

Discussion f method

It is difficult to find the ideal variable for expressing physical work capacity in studies on the elderly. This is due to many factors. It is impossible to determine exactly the reproducibility of the exercise tests and the different ariables used in their interpretation, since repetition of tests at short intervals of time entails the introduction of training stimulating factor the importance of which cannot be measured in the individual case. The maximal physical work capacity can be limited not only by circulatory but also, for example, by respiratory muscular or psychological factors. The importance of these factors in the individual persons cannot be estimated exactly. Special difficulties are thus attached to interpretation of the variable $W_{max\ perf}$. Obviously an attempt was made to carry the exercise test up to the same degree of exhaustion on each test occasion, but this involves subjective factor hich gives rise to some uncertainty. Calculations based on final pulse rate lying at or in the vicinity of biological pulse maximum would seem to give more reliable values than those based on lower final pulse, since in the latter case psychological factors, for example,

Table III *Individual differences in variables used as expressions physical work capacity ($\Delta W = W$ after treatment minus W before treatment)*

Iron group No	ΔW_{130}	ΔW_{150}	ΔW_{170}	$\Delta W_{HR_{max}}$	$\Delta W_{max\ perf}$	ΔHR_{max}	ΔW_{lact}	Placebo group No.	ΔW_{130}	ΔW_{150}	ΔW_{170}	$\Delta W_{HR_{max}}$	$\Delta W_{max\ perf}$	ΔHR_{max}	ΔW_{lact}
♂ 1	+ 50	+ 133	—	+ 135	0	− 9	+ 84	♂ 2	22	− 44	—	− 44	−0	− 4	− 59
3	− 73	− 33	+ 7	+ 7	50	7	+ 35	4	+ 57	+ 11	− 45	− 45	0	+ 2	− 95
5	+ 97	—	—	+ 97	+ 100	+ 15	+ 66	6	− 22	− 9	+ 6	+ 6	0	− 1	+ 6
7	+ 84	+ 64	41	+ 41	0	− 5	14	8	+ 36	+ 8	—	+ 8			—
10	+ 108	+ 138	+ 169	+ 169	+ 200	+ 6	+ 59	9	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	11	+ 107	45	− 19	− 19	0	0	+ 112
14	+ 82	+ 75	—	+ 75	0	− 7	+ 32	13	+ 182	+ 160	+ 138	+ 138	0	− 24	+ 68[b]
15	—	—	—	—	—	—	—	16	+ 8	+ 73	+ 208	+ 208	0	− 10	0[c]
18	+ 17	+ 6	− 6	− 6	0	0	+ 41	17	− 2	+ 8	—	+ 8	0	− 11	+ 61
19	+ 94	+ 104	—	+ 104	+ 50	15	+ 124	20	—	—	—	—	+ 50	− 11	—
22	+ 52	—	—	+ 52	0	8	+ 138	21	+ 15	+ 29	—	+ 29	0	− 4	+ 206
24	[illegible]	+ 27	—	+ 27	+ 200	+ 23	+ 2	23	+ 18	+ 67	—	+ 67	0	− 2	− 42
25	+ 199	+ 186	—	+ 186	+ 100	− 12	+ 154								
♀ 3	+ 33	—	—	+ 33	+ 133	+ 31	− 24	♀ 1	+ 76	+ 20	+ 11	+ 11	0	0	+ 12
4	+ 99	+ 13	—	+ 13	+ 50	+ 2	119	3	+ 30	+ 21	—	+ 21	0	− 4	+ 40
6	− 19	− 14	10	− 10	0	+ 3	+ 13	5	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	7	− 38	− 116	− 226	− 226	− 200	− 8	− 229[d]
9	+ 47	+ 38	+ 29	+ 29	+ 17	− 4	0	10	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	11	+ 10	− 27	—	− 27	0	+ 2	+ 11
13	+ 187	—	—	+ 187	0	+ 7	—	14	+ 53	—	—	+ 53	0	− 7	+ 6
15	+ 41	+ 32	24	+ 24	0	− 6	+ 83	16	+ 14	+ 9	+ 4	+ 4	− 35	0	− 17
18	+ 24	+ 21	+ 18	+ 18	+ 67	+ 15	+ 20	17	+ 3	—	—	+ 3	+ 33	+ 31	+ 33
20	+ 209	+ 190	+ 169	+ 169	+ 167	+ 3	+ 74	19	+ 218	+ 211	+ 116	+ 116	0	− 14	+ 120
21	—	—	—	—	—	—	—	22	+ 84	—	—	+ 84	+ 67	− 4	+ 7

Exercise test not comparable with respect to the variables $W_{max\ perf}$ and HR_{max} on account of lack of co-operation

[b] Interpretation of results of test I difficult. Subject considered to be in an unusually poor condition on this occasion. Had just recovered from protracted upper respiratory infection and also felt depressed owing t death of wife.

Reproducibility calculated on duplicate exercise tests on tw consecutive days before treatment very low Lability in pulse and blood pressure mechanism? Previously periodically treated for stress hypertension.

During period of treatment subject had prolonged infection of respiratory tract, probably of importance for the reduction in physical work capacity

will probably have a greater influence on the heart rate. But even calculations based on a high final pulse rate can be misleading, since the work achievement can have been attained with varying degrees of anaerobic metabolism. If, therefore, the physical work capacity is expressed as a function not only of the highest achieved work intensity but also of the lactate concentration at that point of time, theoretically a better measure of the capacity for oxygen transport should be obtained. Here, however, one further uncertain variable is introduced, especially if the lactate concentration has been determined on capillary blood. Nor are submaximal, indirect measures of physical work capacity (W_{150}, W_{130} and W_{170}) completely satisfactory. It is improbable that determination of W_{150} will allow any good prediction of the maximal physical work capacity in persons whose HR_{max} lies appreciably over 150/min. If W_{150} or W_{170} are chosen, the calculations have to be based to a larger extent on extrapolated values and, in addition, there will be a non-random loss of subjects. In intraindividual comparisons the variable which is to be used (W_{150} etc.) should be calculated in the same way on all test occasions, as an interpolated or extrapolated value. If it is desired to base the comparisons on a variable which will allow reasonably good prediction of the maximal physical work capacity but which does not involve the uncertainties of $W_{max\ pert}$ or W_{lact}, it seems suitable to choose for each individual subject the one of the variables W_{150} etc., which has been calculated on a pulse rate as close as possible to HR_{max} for that subject, and which can be calculated on all the test occasions included in the comparisons (ΔW_{max}).

Table III presents individual differences in the variables which were used as expressions of physical work capacity and also the differences in HR_{max} between the pre- and post-treatment values.

RESULTS

From Table IV *a* and *b* it is evident that

1. Hb and S-Fe did not change;

2. TIBC decreased 10% in the iron group in both sexes, and 2–5% in the placebo group;

3. the degree of saturation of TIBC increased in the iron group by 9% in the men and 20% in the women, and remained essentially unchanged in the placebo group;

4. the non-haemin iron in bone marrow increased in the female iron group by 14% and decreased in the female placebo group by 27%. In the male groups there were only small changes;

5. the changes in THb and TBV were small and showed no distinct tendency;

6. in the iron group the mean values for all W variables increased, in men the increases for the variables W_{130}, W_{150}, W_{170} and $W_{max\ pert}$ varied between 5 and 10% and in women between 10 and 15%. The placebo group also showed an increase, as a rule, in the mean value for these variables: in men an increase varying between 1 and 5% and in women a change varying from a decrease of 3% to an increase of 12%. The mean increase was thus greater in the iron group than in the placebo group (about 12 and 3% respectively). The mean relative increase in the iron group was somewhat smaller for men (about 8%) than for women (about 16%). In the placebo group the difference in the mean relative increase was smaller (about 4% for men and 2% for women). These mean percentage values remained essentially unchanged also when $W_{max\ pert}$ was excluded from the calculations.

7. the difference in HR_{max} in the different groups before and after treatment was small and in itself negligible, but nevertheless this difference should not be disregarded when interpreting $W_{max\ pert}$. It might be argued that the iron treatment could have influenced HR_{max}, but such a causative factor is improbable since the change did not show the same tendency in the two sexes. The trend towards a lower HR_{max} in the placebo group does not seem to be explainable by any similar assumptions and it is probable that the changes can be ascribed to random variation. To what extent and in what way consideration should be taken of these changes in HR_{max} when interpreting $W_{max\ pert}$ cannot be stated unequivocally. When, therefore, W_{lact} was chosen as another measure of submaximal circulation-limited physical work capacity not directly dependent upon the final pulse, a mean increase of about 6% was noted in the iron group and about 2% in the placebo group, i.e. changes which agreed essentially with corresponding calculations based on $W_{max\ pert}$ or submaximal indirect measures of physical work capacity such as W_{130}, W_{150} and W_{170}.

Since the changes for men and women followed mainly the same pattern, the sex groups were combined in the further analyses.

In Table V it can be seen that

1. in the iron group TIBC decreased significantly and for all measures of physical work capacity there was a significant increase;

2. in the placebo group, the mean differences and *p*-values were essentially the same whether the values were calculated on the complete or the reduced group (in which three subjects were

Table IV *a* and *b* *Mean values and standard deviations for the variables studied, before and after treatment in groups according to sex and form of treatment*

Table IV

	Male						Female					
	Iron group			Placebo group			Iron group			Placebo group		
Variables		Mean	S.D		Mean	S.D		Mean	S.D		Mean	S.D.
Hb g%												
Before treatment	*13*	*13.22*	*0.81*	*12*	*13.41*	*0.69*	*10*	*12.67*	*0.71*	*10*	*12.32*	*0.77*
After treatment		13.22	0.93		13.55	0.71		12.76	0.88		12.24	0.65
Difference		0			+0.14			+0.09			−0.08	
S-Fe, mg%												
Before treatment	13	116.6	28.0	12	119.8	23.0	10	95.7	30.4	10	106.6	17.1
After treatment		112.3	48.5		112.9	20.6		98.9	32.6		101.7	43.7
Difference		−4.3			−6.9			+3.2			−4.9	
TIBC, mg%												
Before treatment	13	321.9	48.1	12	326.3	50.9	10	333.2	37.0	10	341.3	44.1
After treatment		289.9	50.4		308.4	48.3		298.0	38.5		333.3	45.5
Difference		−32.0			−17.9			−35.2			−8.0	
Sat. TIBC, %												
Before treatment	13	36.5	9.6	12	37.6	10.0	10	28.5	7.7	10	31.5	5.2
After treatment		39.9	17.1		38.2	12.7		34.2	14.3		30.5	6.4
Difference		+3.4			+0.6			+5.7			−1.0	
Non-haemin iron in bone marrow												
Before treatment	13	22.9	8.2	10	22.5	6.0	10	23.7	5.4	9	20.6	7.6
After treatment		23.8	6.7		23.7	3.9		27.0	6.8		15.0	6.3
Difference		+0.9			+1.2			+3.3			−5.6	
THb, g												
Before treatment	13	667.1	98.7	11	642.7	54.4	10	472.5	72.6	10	494.2	94.0
After treatment		660.8	105.1		606.3	68.5		456.4	74.9		498.9	88.5
Difference		−6.3			−36.4			−16.1			+ 4.7	
BV l												
Before treatment	13	5.65	0.82	11	5.23	0.50	10	4.09	0.52	10	4.41	0.81
After treatment		5.54	1.08		4.91	0.59		3.93	0.64		4.47	0.74
Difference		−0.11			−0.32			−0.16			+0.06	

excluded) Only for W_{130} when calculated on the complete group was there a significant mean difference, viz. an increase ($0.01 < p < 0.02$)

3 the differences in mean differences between iron group and placebo group were significant for $\Delta W_{max\ part}$ when calculated for the complete and the reduced series ($0.001 < p < 0.01$ and $0.01 < p < 0.02$) and for $\Delta W_{II\ max}$ when calculated on the reduced placebo group ($0.02 < p < 0.05$) and almost reached the significant level when calculated on the complete placebo group ($0.05 < p < 0.1$) For the remaining ΔW variables the p-values for the differences between the mean differences were somewhat higher

Fig. 3 shows Hb before and after treatment. The change in Hb was not correlated to the initial Hb value. Thus there was no tendency for subjects with a relatively low initial Hb to higher values after the iron treatment.

Fig. 4 shows S-Fe before and after treatment Subjects with a low initial S-Fe showed no grea tendency to increase their S-Fe value after trea ment than other subjects.

Fig. 5 shows TIBC before and after treatm In the iron group eight subjects had a very sl increase in TIBC and 15 a decrease, which general was considerably larger In the place group seven subjects showed an increase in and 15 a decrease. The numerical decrease TIBC was greater in the iron than in the p group

Fig. 6 shows the degree of saturation of t before and after treatment. In the iron group subjects showed an increase in saturation eight a decrease. In the placebo group the

Table IV *b*

Variables	Male: Iron group			Male: Placebo group			Female: Iron group			Female: Placebo group		
		Mean	S.D.		Mean	S.D.		Mean	S.D		Mean	S.D
W_{130}, kpm/min												
Before treatment	11	652.8	130.6	10	584.9	116.2	8	320.4	113.5	9	365.7	112.0
After treatment		717.1	152.9		622.6	127.5		398.0	145.3		410.1	78.2
Difference		+64.3			+37.7			+78.4			+44.4	
W_{150}, kpm/min												
Before treatment	9	845.2	158.9	10	761.1	134.4	6	413.5	130.0	6	518.0	136.8
After treatment		923.0	167.3		795.9	130.7		460.2	137.0		537.7	101.1
Difference		+77.8			+34.8			+46.7			−19.7	
W_{170}, kpm/min												
Before treatment	4	1 007.2	182.2	5	877.2	145.8	5	480.4	105.5	4	670.7	160.1
After treatment		1 060.2	222.7		934.8	178.3		526.4	127.8		647.0	101.7
Difference		+52.8			57.6			+46.0			−23.7	
$W_{max\,pert}$, kpm/min												
Before treatment	11	845.4	154.4	10	825.0	120.9	8	464.6	83.6	9	551.9	116.6
After treatment		900.0	187.1		830.0	118.7		518.9	91.0		537.0	80.8
Difference		+54.6			−5.0			+54.3			−14.9	
HR_{max}, beats/min												
Before treatment	11	156.4	10.6	10	161.1	7.5	8	153.7	13.1	9	154.3	13.0
After treatment		154.7	9.4		154.6	9.0		160.1	14.2		153.9	16.1
Difference		−1.7			−6.5			+6.4			0.4	
$W_{HR\,max}$, kpm/min												
Before treatment	11	645.6	131.0	9	672.2	154.5	7	410.7	105.9	9	440.1	90.5
After treatment		712.1	112.2		700.8	176.7		417.4	99.6		438.2	72.0
Difference		+66.5			−28.6			+6.7			1.9	

responding figures were nine and 13 respectively.

Fig. 7 shows non-haemin iron in the bone marrow before and after treatment. In the iron group this increased in 13 subjects, remained unchanged in two, and decreased in eight. The corresponding figures in the placebo group were nine, one and nine. The figure shows no tendency for the change in non-haemin iron in the marrow to be dependent upon the initial value.

Fig. 8 shows that, in all subjects in whom W $_{30}$ decreased during the period of treatment, the treatment was terminated during January–February. The mean value for W_{130} after treatment was approximately the same for the group where treatment was terminated before March 31 and for the group where treatment was terminated during the period April 1 to June 30. The mean value for ΔW_{130} was somewhat higher for the latter group: the difference, however, was insignificant ($0.05 < p < 0.1$). When $\Delta W_{HR\,max}$ was used as an index of change in physical work capacity, the difference between the groups was significant ($0.01 < p < 0.02$). As the subjects from the iron and placebo groups were evenly distributed over the whole investigation period, the seasonal variation in physical work capacity had

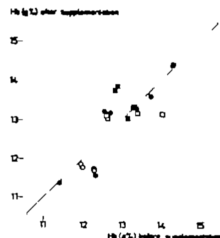


Fig. 5. Hb concentration before and after treatment. ■ ● iron group, men and women, respectively; □ ○ placebo group, men and women, respectively.

Table V. *Mean differences for the variables studied, calculated as the value after treatment minus the value before treatment, standard error of the mean for the mean differences, degrees of significance for these mean differences, differences in mean differences between the iron group and the placebo group and degrees of significance for these differences*

Variables	Iron group				Placebo group				Differences in mean differences between iron group and placebo group	
		Mean diff.	S.E.M.	p		Mean diff.	S.E.M.	p	Diff	p
ΔHb g %	23	− 0.03	0.15	0.8 < p < 0.9	22	− 0.04	0.15	0.8 < p < 0.9	+ 0.01	0.9 < p
ΔS-Fe, μg	23	− 1.0	6.8	0.8 < p < 0.9	22	− 6.0	6.0	0.3 < p < 0.4	+ 5.0	0.5 < p 0.6
ΔTIBC, μg	23	− 32.0	11.3	0.001 < p < 0.01	22	− 13.9	14.60	0.3 < p < 0.4	− 18.1	0.3 < p < 0.4
ΔSat, TIBC,	23	+ 4.4	2.7	0.1 < p < 0.2	22	− 0.2	2.4	0.9 < p	+ 4.6	0.2 < p < 0.3
ΔNon-haemin iron in bone marrow	23	+ 1.7	1.5	0.2 < p < 0.3	19	− 2.1	2.3	0.3 < p < 0.4	+ 3.8	0.1 < p 0.2
ΔTHb g	23	− 14.1	12.2	0.2 < p < 0.3	21	− 16.9	15.1	0.2 < p < 0.3	+ 2.8	0.8 < p 0.9
ΔTBV l	23	− 0.130	0.12	0.2 < p < 0.3	21	− 0.136	0.144	0.3 < p < 0.4	− 0.06	0.7 < p 0.8
ΔW[illegible] kpm/min	19	+ 69.9	16.7	p < 0.001	19	+ 40.9	15.3	0.01 < p < 0.02	+ 28.0	0.2 < p < 0.3
					16[a]	+ 36.8	14.0	0.02 < p < 0.05	+ 33.1	0.1 < p < 0.2
ΔW[illegible] kpm/min	15	+ 65.3	18.1	0.001 < p < 0.01	16	+ 29.1	18.9	0.1 < p < 0.2	+ 36.2	0.1 < p < 0.2
					13[a]	+ 26.8	17.2	0.1 < p < 0.2	+ 38.5	0.1 < p < 0.2
$\Delta W_{HR\,max}$ kpm/min	19	+ 70.9	15.6	p < 0.001	19	+ 20.9	20.2	0.3 < p < 0.4	+ 50.1	0.05 < p 0.1
					16[a]	+ 17.2	11.1	0.1 < p < 0.2	+ 53.8	0.02 < p 0.05
$\Delta W_{max\,perf}$, kpm/min	19	+ 54.4	17.2	0.001 < p 0.01	19	− 4.4	12.0	0.7 < p < 0.8	+ 58.8	0.001 < p 0.0[illegible]
					16[a]	+ 6.1	5.9	0.3 < p < 0.4	+ 48.3	0.01 < p < 0.02
ΔHR_{max}, beats/min	19	+ 1.7	2.8	0.1 < p < 0.2	19	− 3.0	2.7	0.2 < p < 0.3	+ 4.7	0.2 < p < 0.3
					16[a]	1.7	2.6	0.5 < p < 0.6	+ 3.4	0.3 < p < 0.4
ΔW_{lact}, kpm/min	18	42.7	15.3	0.01 < p < 0.02	18	+ 13.3	21.7	0.5 < p < 0.6	+ 29.3	0.2 < p 0.3
					15[a]	+ 26.7	19.4	0.1 < p < 0.2	+ 15.9	0.6 < p < 0.6

[a] Subjects nos. ♂ 13, 16 and ♀ 7 excluded (cf. footnote Table III).

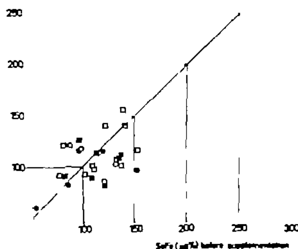


Fig. 4. Serum iron before and after treatment. ■ ● group men and women, respectively; □ ○ placebo men and women, respectively.

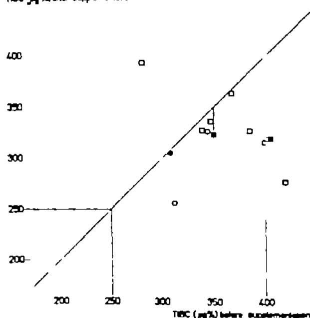


Fig. 5. Total iron binding capacity before and after treatment ■ ● iron group, men and women, respectively; □ ○ placebo group, men and women, respectively.

no influence on the comparison between iron and placebo groups.

Table VI gives coefficients of correlation between the changes in physical work capacity and (*a*) the initial values of and (*b*) the changes in Hb, S-Fe, TIBC, degree of saturation of TIBC, and non-haemin iron in the bone marrow. In the iron group there were only weak, non-significant correlations. In the placebo group there was a negative correlation between increase in physical work capacity and change in non-haemin iron in the marrow and a positive correlation between increase in physical work capacity and the initial value for non-haemin iron in the bone marrow. These correlations were significant ($0.01 < p \leq 0.5$). Figs. 9 and 10 illustrate these relationships.

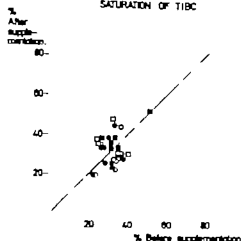


Fig. 6. Saturation of TIBC before and after treatment ■ ● iron group, men and women, respectively; □ ○ placebo group, men and women, respectively.



Fig. 7. Non-haemin iron in bone marrow before and after treatment ■ ● iron group, men and women, respectively; □ ○ placebo group, men and women, respectively.

Table I *Arrhythmias treated with alprenolol in a series of patients with coronary heart disease*

Age (y)	Arrhythmia	Total dose of lprenolol (mg)	Result	Complications
81	Ventricular tachycardia	2.5	None	Hypotension and shock
62	Atrial flutter	10	Reduction of ventricular rate	—
52	Atrial fibrillation	10	Reduction of entricular rate	—
51	Atrial fibrillation	10	Reduction of ventricular rate	—
43	Atrial fibrillation	10	Sinus rhythm	—

RESULTS

Tables I and II present the results obtained in patients with and without recent myocardial infarction.

Ventricular ectopies

The two patients with ventricular ectopic beats of over five per min, and one patient with bi geminy not digitalis-induced, responded satisfac torily to the treatment (Table II). The other case of bigeminy proved to be refractory to 10 mg of alprenolol, but the arrhythmia was readily elimi-nated with 25 mg of lignocaine. There was an average 20 mmHg decrease of systolic blood pres re in these patients without other side effects.

Atrial flutter and fibrillation

Sinus rhythm was achieved in only one of the three patients with atrial fibrillation and high ventricular rate (Table I) all digitalized In the two other cases a significant reduction of ven tricular rate by an average of 30% was achieved No side effects were noted apart from a sl decrease in the systolic blood pressure The pa tient with flutter and atrial frequency of 280/m with 2 1 block showed a result of 4 1 bk and ventricular rate of 72/min after 10 mg o alprenolol.

Ventricular tachycardia

Of the three patients with ventricular tachycardia the one without infarction (Table I) showed hy tension with clinical signs of shock after 2.5 m of alprenolol No effect on the tachycardia of 156/min was observed The hypotension wa readily corrected with metaraminol-noreph infusion, and DC shock, 100 ws, resulted in sin rhythm

One of the two patients with myocardial ir farction (Table II) had an initial heart rate c 148/min and blood pressure of 120/90 mml

Table II *Arrhythmias treated with alprenolol in a series of patients with recent myocardial infarction*

Age (y)	Arrhythmia	Total dose of alprenolol (mg)	Result	Complications
54	Ventricular tachycardia	5.0	None	Hypotension and shock
46	Ventricular tachycardia	2.5	None	—
61	Supraventricular tachycardia	2.5	None	Shock
63	Supraventricular tachycardia	5.0	Sinus rhythm	—
44	Supraventricular tachycardia	5.0	Sinus rhythm	—
69	Supraventricular tachycardia	2.5	Sinus rhythm	Shock
61	Ventricular ectopic beats	5.0	Sinus rhythm without ectopic beats	—
62	Ventricular ectopic beats	5.0	Sinus rhythm without ectopic beats	—
55	Ventricular bigeminy	10.0	Sinus rhythm without ectopic beats	—
56	Ventricular bigeminy	10.0	None	—

The administration of 5 mg of the drug was followed by a sudden circulatory collapse. The blood pressure fell to an unmeasurable level, the patient became pale and cyanotic, and vomited. DC shock, 100 ws, resulted in sinus rhythm. The blood pressure was restored with metaraminol infusion, and after 20 min the original level was obtained.

In the third patient with ventricular tachycardia of 166 beats per min no effect was obtained after 2.5 mg of the drug. Because of the experiences described above, DC shock, 100 ws, was used and resulted in sinus rhythm.

Supraventricular tachycardia

All four patients had recent myocardial infarction (Table II). The injection of 2.5 mg of alprenolol in a patient of 61 years with inferior wall infarction and a heart rate of 182/min was followed by a sudden circulatory collapse without effect on the heart rate. Prior to the injection the patient was in good clinical condition. No clinical or radiological signs of left ventricular failure were seen, and his blood pressure was 130/90 mmHg. A DC countershock given immediately afterwards restored the sinus rhythm, and the blood pressure was readily restored with norepinephrine infusion. The tachycardia relapsed four times and remained stable only after intravenous procainamide.

In two patients 5 mg of alprenolol resulted in sinus rhythm without complications, and the effect was maintained with oral alprenolol, 50 mg four times a day.

The fourth patient with an initial heart rate of 176/min and blood pressure of 110/90 mmHg had moderate clinical and roentgenological evidence of left ventricular failure. The patient was digitalized with no effect on the heart rate. An injection of only 2.5 mg of alprenolol resulted in sudden, deep clinical shock, followed secondarily by cardiac arrest.

Extrathoracic cardiac massage and artificial ventilation with pure oxygen were used and resulted in a sinus rhythm of 106 beats per min. The blood pressure, restored with metaraminol-norepinephrine infusion, remained stable after two hours, and the patient was saved.

Because of these serious complications the trial was terminated after altogether 15 patients had been treated with the drug.

DISCUSSION

Alprenolol, which differs from propranolol by its intrinsic stimulating effect on the beta-receptor (16), would always maintain a certain basal sympathetic tone on the heart, thereby diminishing the risk of heart depression due to a total elimination of the sympathetic drive. To this effect has been ascribed the fact that 10 mg of alprenolol did not reduce the cardiac output in healthy resting subjects, whereas propranolol did by an average of 23% as shown in a double-blind cross-over study (6).

On the grounds presented above it seems quite logical to suggest that alprenolol has the same antidysrhythmic action combined with a higher degree of safety than propranolol. For these reasons it seems that alprenolol should be a more suitable antiarrhythmic drug in the early stages of myocardial infarction.

Prior to starting the present study there was only one report concerning the intravenous use of alprenolol in acute arrhythmia (11). Of the 43 patients reported, 14 had myocardial infarction. The intravenous dose ranging from 4 to 20 mg was usually well tolerated. However there was a fall in systolic blood pressure of 10–40 mmHg in general, and in three cases clinical signs of shock. In one case sudden circulatory collapse and deep clinical shock followed the injection of 6 mg of alprenolol in a woman without organic heart disease. The conclusion was, however that the drug proved to be an effective anti-arrhythmic agent and could safely be recommeded for clinical work.

The results obtained in the present series as regards the anti-arrhythmic efficacy of the drug do not differ from those so far reported on the use of propranolol. In the light of the high incidence of complications, four among 15 cases, it is evident that the slight stimulating effect of the drug on the beta-receptor is not sufficient to compensate for its blocking action. Therefore alprenolol should be considered dangerous in acute myocardial infarction. The findings of Saumamäki and Pedersen (13) in a material of seven patients were quite similar: in three out of seven patients pronounced reduction in blood pressure was obtained.

Recently Lund Larsen and Sivertssen (12), in comparative study of the haemodynamic effects

of propranolol and alprenolol in the acute stage of myocardial infarction, have clearly shown that cardiac index, heart rate and stroke index were significantly reduced after both drugs. They concluded that both drugs should be considered dangerous in patients in whom augmented sympathetic drive is necessary for the maintenance of adequate cardiac contractility and rate

The mechanisms involved in the acute fall in blood pressure are, according to general opinion, ascribed to the negative inotropic effect of the drugs. However it should be borne in mind that alprenolol and propranolol are not cardioselective drugs. The peripheral actions might also be of importance in these situations.

Grayson (7) has pointed out that the danger of a beta-blocker lies in its action to potentiate the vasoconstrictor effects of adrenaline and noradrenaline. Once the beta-receptors which mediate vasodilation are blocked, adrenaline, through its action on alpha-receptor becomes a most powerful vasoconstrictor agent—and noradrenaline even more so He considers the administration of adrenaline and noradrenaline to be strongly contraindicated in propranolol-treated patients. However all the present patients with shock were treated with metaraminol noradrenaline or both, d the blood pressure was readily restored with- complications.

In the light of the hitherto negative experiences with beta-blockers in acute myocardial infarction, it is interesting to note the excellent results reported by Jewitt et al. (8) They used a cardioselective beta-adrenergic blocking drug, practolol, for the treatment of arrhythmias in the acute phase of myocardial infarction. The anti-arrhythmic action of practolol was especially valuable in supraventricular arrhythmias associated with rapid heart rate and haemodynamic deterioration It should be noted that several lignocaine resistant ventricular tachycardias were readily corrected with this drug. The experience that practolol did not precipitate or aggravate heart failure in any of these patients indicates a distinct advantage over the beta-blockers hitherto used.

This might be explained by the drug's cardioselective action, slight sympathomimetic properties and absence of local anaesthetic, i.e. a direct cardiodepressive action.

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EFFECT OF INTRAVENOUS PROSTAGLANDIN E_1 ON NORADRENALINE STIMULATED MOBILIZATION OF PLASMA FREE FATTY ACIDS IN MAN

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Abstract. Prostaglandin E_1 (PGE_1) has been given intravenously during continuous noradrenaline infusion into healthy male subjects. Control experiments were also performed in which saline was given instead of PGE_1. There were four subjects in each group. Noradrenaline was given at a rate of 0.1 μg/kg/min i.v. during 210 min. When noradrenaline had been given for 80 min, PGE_1 was infused simultaneously in three doses, each during 30 min, the dose being successively increased from 0.10 μg/kg/min to 0.18 and finally to 0.32 μg/kg/min. The PGE_1 infusion induced a flush in all subjects. Three of the four subjects given PGE_1 had headache and two abdominal cramps. In one of the subjects the PGE_1 infusion with the highest dose, 0.32 μg/kg/min, had to be stopped after 25 min because of respiratory distress. The noradrenaline infusion increased the blood pressure with a slight decrease in heart rate. When PGE_1 was infused simultaneously the blood pressure decreased and the heart rate increased. The concentration of free fatty acids (FFA) in blood plasma increased markedly during the noradrenaline infusion. This rise in FFA concentration was not significantly influenced by the simultaneous administration of PGE_1. The fact that no inhibition of FFA mobilization could be demonstrated does not rule out a physiological effect of PGE_1 in this regard, but suggests that PGE_1 is then not transported by the blood to adipose tissue.

In 1963 Steinberg et al. (12) demonstrated that prostaglandin E_1 (PGE_1) inhibits the basal as well as the catecholamine-stimulated lipolysis in rat epididymal adipose tissue in vitro. Further studies showed that PGE_1 also inhibits the catecholamine-stimulated mobilization of free fatty acids (FFA) in vivo in the dog (3, 4, 5, 13).

PGE_1 was found to inhibit lipolysis also in human adipose tissue in vitro, both in subcutaneous and omental tissue of male as well as of female origin (1, 10). However, when PGE_1 was infused to man in vivo there was unexpectedly an *increase* in FFA mobilization (2, 7). Furthermore, when PGE_1 was given together and simultaneously with noradrenaline intravenously into man, there was no significant reduction of the catecholamine-stimulated rise of FFA concentration. This suggested that intravenous infusion of PGE_1 was not effective in inhibiting the increased FFA mobilization induced by catecholamines in man in vivo, at least under the conditions used.

This investigation was performed to further study the possible influence of PGE_1 on noradrenaline-stimulated FFA mobilization in man by another technique. An infusion of noradrenaline was started, and PGE_1 was then given for a period during the continuous infusion of catecholamine. For comparison control experiments were performed in which saline was given instead of PGE_1.

MATERIAL AND METHODS

Procedure

Five male volunteers, between 21 and 26 years of age, were studied, three of them twice (with and without PGE_1). They were healthy as judged from routine clinical and laboratory investigation. Exercise tests did not show any abnormalities with respect to systemic circulation or ECG (11).

The subjects reported at the laboratory at 8 a.m. after fasting overnight. One catheter of teflon was placed percutaneously into the brachial artery after local anesthesia with Carbocain® (Bofors, Sweden). Two teflon catheters were introduced into the veins of the opposite arm for infusion of PGE_1 and noradrenaline. With all catheters in place the subjects rested comfortably in the supine position throughout the study. Noradrenaline was given as an i.v. infusion at a constant rate of 0.1 μg/kg/min

Table I. *Clinical effects of PGE during continuous infusion of noradrenaline 0.1 μg/kg/min*

0 = no symptoms. F = flush. H = headache. Rest. = restlessness. Resp. = respiratory distress. Pr = pressure in the chest. A = bdominal cramps. Pal. = pallor

Subject	Infusions	Infusion rate of PGE μg/kg/min: 0	0.10	0.18	0.32
J T	Noradren. PGE	0	F H	F H Rest., Cough	F H Resp., Pr Cough
	Noradren.	0	0	0	0
A. V	Noradren. + PGE	0	F Resp.	Rest. F	~H Pal., A, Rest.
	Noradren.	0	0	0	0
C. T	Noradren. + PGE_1	Pr	F Resp.	Rest F	H Pal., A, Rest.
	Noradren.	H	0	0	0
B. U	Noradren. PGE	0	0	F	F Rest.
R. S.	Noradren.	Pr	0	0	0

during 210 min. The PGE_1 infusion was started after 80 min and given during 90 min at increasing rates of 0.10, 0.18 and 0.32 μg/kg/min, each rat during a 30 min period. Three subjects received PGE_1 and noradrenaline, and after some weeks the experiment was repeated with noradrenaline only (control experiments). Two subjects were studied only once one was given PGE_1 and nor adrenaline, and the other noradrenaline and saline (control experiments).

The arterial blood pressure was recorded continuously with an Elema differential transformer transducer (EMT 490 A). The pressure was calculated over several respiratory cycles, and the mean pressure was obtained by electrical integration (time constant 0.8 sec). Heart rat was calculated from an ECG recording.

Prostaglandin E was obtained from Professor S. Bergström, as a crystalline preparation. PGE_1 dissolved in saline was sterilized by ultrafiltration. This sterile solution of PGE_1, containing 50 μg/ml, was dispensed in 5 ml portions and stored at −15°C. The solution was diluted in 0.9% saline immediately before the infusion. Noradrenaline was received from Astra (Sweden) as Noradrina® conc.

Analysis

Arterial blood was withdrawn into heparinized syringes. No heparin was injected, and 0.9% saline was used for flushing f the catheters. Aliquots of blood ere precipitated for determination of glucose (9) and the remainder was promptly centrifuged to separate cells from

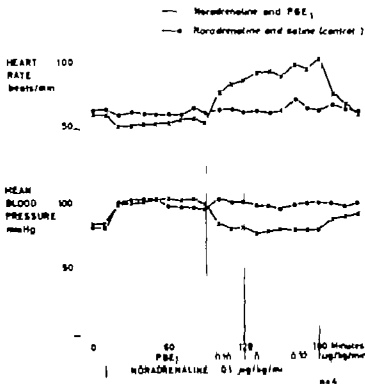


Fig. 1. Effect of PGE_1 on heart rate and mean blood pressure during continuous infusion of noradrenaline in man. Noradrenaline infused at constant rate of 0.1 μg/kg/min. PGE_1 given at three different rates, 0.10, 0.18 and 0.32 μg/kg/min, each dose during 30 min as indicated in the figure. Mean values from studies on four healthy subjects.

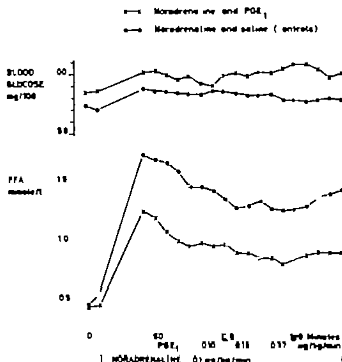


Fig. 2. Effect of PGE_1 on blood glucose and free fatty acids (FFA) in blood plasma during continuous infusion of noradrenaline in man. Noradrenaline infused i.v. at a constant rate of 0.1 µg/kg/min. PGE_1 given i.v. at three different rates, 0.10, 0.18 and 0.3? µg/kg/min, each dose during 30 min as indicated in the figure. Mean values from studies on four healthy subjects.

plasma. The plasma was immediately processed and FFA were determined according to Dole (8) with the modification described by Trout et al. (14).

RESULTS

Clinical Effects (Table I)

Noradrenaline. When the noradrenaline infusion had been started, one of the subjects had a transient oppression in his chest before he received PGE_1. In one of the control experiments the subject complained of headache, and in another experiment, pressure in the chest, but otherwise the noradrenaline infusion produced no subjective symptoms.

PGE, produced the usual symptoms seen during i.v. infusion to man (for comparison see Carlson, et al. (7)). Flush was seen in all subjects and sometimes changed to pallor when the higher doses of PGE_1 were given. Restlessness and tensions in the body were felt by all four subjects. Three of them had headache and two abdominal pains. One of the subjects complained of severe respiratory distress and the infusion of PGE_1 had to be stopped after 25 min with 0.3 µg/kg/min. The various symptoms had almost completely disappeared at the time when the infusion of noradrenaline was stopped.

Effects on Blood Pressure and Heart Rate (Fig. 1)

The infusion of noradrenaline increased the blood pressure in all subjects, systolic pressure from an average of 118 mmHg to a level of 140 mmHg in the control group and to 15? mmHg in the PGE_1-group. The mean pressure increased from 86 mmHg to 104 mmHg and 107 mmHg respectively. In the control group the blood pressure was elevated during the whole period of noradrenaline infusion. Infusion of PGE_1 immediately induced a fall in blood pressure. The systolic level during the three periods with the increasing dose of PGE, was 141, 133 and 1?7 mmHg respectively. When the infusion of PGE, was stopped (noradrenaline infusion being continued), the blood pressure increased. The noradrenaline infusion alone lowered the heart rate from a mean of 64 to 49 beats/min. When the infusion of PGE, was started, the heart rate increased in all subjects. The mean frequency was 54 beats/min before PGE_1 infusion and 87, 96 and 10? beats/min during each of the three periods with PGE, respectively. When the PGE, infusion was stopped, the heart rate decreased to values similar to those in the control group within 30 min.

Table II. *Concentration of FFA in subjects given noradrenaline (Nor) with and without PGE₁ as indicated in Fig. 2*

	Before Nor infusion (mmol/L)	During Nor infusion (per cent of conc. before Nor) 40′	80′	110′	140′	170′	After Nor infusion 30′
Nor + saline (n = 4)	0.55	314	266	252	259	246	280
Mean ± S.E.	0.08	17	9	12	18	33	41
				+ PGE₁ infusion 30′	60′	90′	
Nor + PGE₁ (n = 4)	0.47	279	210	217	193	190	208
Mean ± S.E.	0.06	40	18	19	21	21	31

Metabolic Effects (Fig. 2 and Table II)

The noradrenaline infusion increased the concentration of FFA in all subjects, after 40 min to 314% of the basal value in the control group and to 279% in the group later receiving PGE_1. The basal values as well as the rise in the PGE_1 group were evidently numerically smaller but the difference was not statistically significant. Before the PGE_1 infusion started, the FFA level decreased to a new level around 200% and this level was almost unchanged throughout the study. The changes (mean ± S.E.) in FFA concentration after 30, 60 and 90 infusion of PGE_1 were $+0.02 \pm 0.06$, -0.10 ± 0.08, and -0.11 ± 0.12, respectively. The corresponding figures in the control group were -0.10 ± 0.08, -0.11 ± 0.12, and -0.18 ± 0.20. It is thus clear that PGE_1 did not to any major extent modify the FFA mobilizing effect of noradrenaline.

The noradrenaline infusion increased the blood glucose level in all subjects. The mean rise in the control group was from 70 to 88 mg per 100 ml, and in the PGE_1 group from 86 to 102 mg per 100 ml. As is obvious from Fig. 2, the mean level of blood glucose was numerically higher in the PGE_1 group throughout the study but the difference was not statistically significant.

DISCUSSION

The clinical effects of PGE_1 were of similar type to those previously reported (7). Apparently the previous and the simultaneous administration of noradrenaline does not modify these effects of PGE_1.

An inhibitory effect of infused PGE_1 on the noradrenaline-induced rise in FFA concentration in the dog has been demonstrated with a similar technique as used in this study (4). Turnover rate studies with labelled palmitic acid clearly showed that this decrease in FFA concentration in the dog was due to an inhibition of the noradrenaline-induced FFA mobilization. In this study in man, however, the PGE_1 infusion did not influence the noradrenaline-induced rise in FFA concentration, which is in agreement with our previous study in man (7), when a somewhat different technique was used.

These findings may appear somewhat surprising, since PGE_1 in vitro has a marked inhibitory effect on catecholamine as well as basal lipolysis in adipose tissue from man (1, 10). There are several possible explanations of this discrepancy in the results from dog and man. Species differences may be of importance. In the dog the infused dose of PGE_1 was somewhat higher, around 0.4 µg/kg/min, which is usually not tolerated in man because of the clinical effects, like abdominal cramps, tensions, headache, and so on (7). However, to judge from the similarity of the blood pressure effect, the dose used in this study in man is at least in that regard comparable to the dose previously used in the dog (4).

Furthermore the experiments in the dog were performed on anesthetized animals. This fact may be of great importance, as PGE_1 infusions also have a stimulatory effect on FFA mobilization (5). This effect appears to be due to an increased sympathetic nervous activity. As anesthesia with pentobarbital (5) is known to depress the sympathetic nervous activity, it is possible that the inhibitory effect of PGE_1 infusions on FFA mobilization is more pronounced than the stimulatory effect in the anesthetized animal. In comparison

to the non-anesthetized dog or man with the dose levels used. The interpretation of the effects of PGE_1 on FFA is however difficult, as the same doses of PGE_1 as used here raised FFA levels when infused into fasting male subjects (7). While, on the one hand, PGE_1 had no inhibitory effect in this study on noradrenaline-stimulated FFA mobilization, there was, on the other hand, no increase in FFA levels. Whether two such effects could mask each other is not known. Although we have not been able to demonstrate any inhibitory effect of infused PGE_1 on the noradrenaline-stimulated FFA mobilization in man it is still possible from the in vitro experiment (1 10) that PGE_1 and/or other prostaglandins have a physiological role within the adipocytes as local inhibitors or modulators of FFA mobilization (6). It seems very unlikely however considering the clinical effects, that PGE_1 normally influences FFA mobilization by being transported in the blood to adipose tissue.

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PLASMA RENIN ACTIVITY IN PATIENTS WITH DISTURBED SYMPATHETIC VASOMOTOR CONTROL (POSTURAL HYPOTENSION)[1]

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Abstract. In three patients with postural hypotension the effect of tilting on plasma renin activity (PRA) has been studied. PRA increased slightly in two patients and decreased in one. Intravenous noradrenaline infusion to one patient studied did not influence PRA.

The mechanisms regulating renin secretion still remain obscure. Experimental studies in dogs (6, 11, 17, 19) and rats (4, 16) suggest that the sympathetic renal nerves play an important role in regulation of renin release.

In man indirect evidence also supports a role for the sympathetic nervous system in regulating renin secretion. For example stimulation of the sympathetic nervous system induced by upright posture (8, 18) or exercise (7, 10) increases plasma renin activity. Furthermore in a study by Gordon et al. (9) it was found that one patient with disturbed function of the sympathetic system (postural hypotension) showed no increase in plasma renin in response to upright posture.

In this report the orthostatic effect on plasma renin activity was studied in three patients with postural hypotension by means of head-up tilting. In one of them plasma renin response to norepinephrine infusion was also studied.

MATERIAL

Two men and one woman with postural hypotension were studied. All of them showed the typical picture of postural hypotension with prompt fall in arterial pressure and no or slight increase in heart rate on head-up tilting and during exercise (Table I). Exercise test performed according to Sjöstrand (14).

Two of them (cases 1 and 3) had previously attended the Department of Endocrinology; case 2 had previously been studied by Bevegård (2) at the Laboratory of Clinical Physiology, Thorax Clinic, Karolinska Sjukhuset. Orö (13) had studied their metabolism of plasma free fatty acids and they were then reported as cases 1, 5, 4 respectively.

CASE REPORTS

Case 1

A 50-year-old man with attacks of fainting since 1950. When he attended the Department of Endocrinology in 1955 his blood pressure was 160/105 mmHg and the heart rate about 80 beats/min before head-up tilting. The urinary excretion of noradrenaline at rest was then 2.8 μg/min. During 3 hours' tilting at 25° the blood pressure was about 80/55 mmHg and the pulse rate did not change. The excretion of noradrenaline was 2.0 μg/min and the excretion of adrenaline was not measurable. Sundin (15) found that tilting of healthy subjects from recumbency to 75° induced a rise in the urinary noradrenaline secretion from 9.8 ± 1.4 at rest to 34.1 ± 3.7 μg/min. The output of adrenaline increased from 1.9 ± 0.4 to 9.5 ± 1.3 μg/min. Before the present study the patient was generally unable to walk more than 25–50 m before he had to sit down to avoid fainting.

Serum creatinine was now elevated to 2.3 mg%, but urine contained no bacteria, cells or protein.

Case

A 77-year-old female known to have hypertension from 1945. In the supine position the blood pressure was 220–200/110–100 mmHg. From 1960 she had attacks of fainting. She attended the Department of Neurology, Serafimerlasarettet, in 1961, where the diagnosis postural hypotension was established. At rest the urinary excretion of noradrenaline was ..3 μg/min and the excretion of adrenaline 1.8 μg/min. Before and during insulin-induced hypoglycemia the noradrenaline excretion

[1] Reported at the Meeting of the Swedish Society of Internal Medicine and the Swedish Society of Endocrinology, Stockholm, March 1968.

Table I. *Effect of exercise on heart rate and systolic blood pressure in the subjects with postural hypotension*

Case no.		Rest	Exercise 1–4 min	7–12 min	13–18 min	Rest 5–10 min
1	Work load, kpm/min	—	150	250	350	—
	Heart rate, beats/min	74	79	83	85	
	Syst. B.P mmHg	130	100	95	95	
2	Work load, kpm/min	—	150	150	150	—
	Heart rate beats/min	83	101	110	115	88
	Syst. B.P mmHg	240	185	185	185	185
3	Work load, kpm/min	—	200	300	400	—
	Heart rate, beats/min	52	57	57	59	56
	Syst. B.P mmHg	140	110	105	100	

values were 4.4 mμg min and 2.. mμg min, respectively. The corresponding figures for adrenaline were ..8 and ..2 mμg min, respectively. The noradrenaline and adrenaline excretion were respectively 2.2 and 1.1 mμg min before and 5.0 and 1.7 mμg min after the administration of histamine.

At the time of the present study she was unable to walk more than 10–30 m. During an exercise test on a bicycle ergometer in the supine position there were ECG changes suggestive of coronary insufficiency at 150 kpm min. The kidney function was normal as judged from routine examinations.

Case 3

70-year-old man with symptoms of postural hypotension for more than ten years. At the time of the

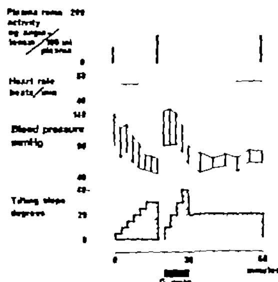


Fig. 1. Effect of tilting on heart rate, blood pressure and PRA in case 1. A G-suite was inflated in the first part of the second tilting.

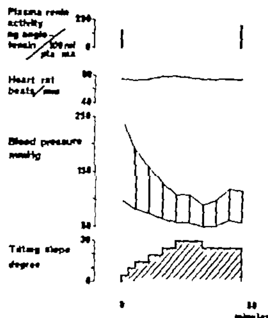


Fig. Effect of tilting on heart rate, blood pressure and PRA in case 2.

present study vision was dimmed after walking 5–10 m and he then had to resort to squatting to avoid fainting. On standing his blood pressure fell from the resting level around 140–110/80–60 to 80–60–40 within 20–30 sec. The heart rate usually remained unchanged at a frequency of 45–47 beats min. When he attended the Department of Endocrinology they had found normal thyroid function with PBI of 5.6 μg%. Normal adrenal function according to clinical and laboratory findings, with daily urinary excretion of 17-ketosteroids of 9–10 mg and 17-ketogenic steroids of 13–16 mg 24 h and with proper increase after ACTH infusion. At bed rest urinary catecholamines were normal, noradrenaline ranging from 7–11 μg and adrenaline 4–6 μg 4 h, but there was no increase in urinary catecholamines in erect position.

There was no evidence of kidney disease. Serum creatinine was 1.1 mg 100 ml and the urine was free from protein.

PROCEDURE AND METHOD

No medication had been given to the patient during the 48 hours before the study. One teflon catheter was inserted into a cubital vein without anesthesia. After at least 30 min on a tilt table the patients were tilted in the head-up position. The tilting angle was slowly increased and adjusted to produce a significant but tolerable blood pressure fall during 30–40 min. (In one patient using a G-suite (case 1), tilting was performed twice with and without the G-suite inflated.) The blood pressure was measured over the brachial artery with sphygmomanometer cuff. The heart rate was estimated from the radial artery pulse. Peripheral venous blood samples were withdrawn into heparinized tubes and immediately cooled in ice water. Plasma renin activity

(PRA) was determined by the method of Boucher et al. (3) with some modifications. PRA is expressed as ng angiotensin/100 ml plasma. For details see Centenfors (7). Normal levels in men in supine position are 30–170 ng/100 ml.

In one patient (case 3) in separate experiment an intravenous infusion of norepinephrine with motor syringe was also performed. The dose of norepinephrine was successively increased from 0.0007 to 0.0027 μg/kg/min during 40 min to induce significant blood pressure rise.

RESULTS

In case 1 (Fig. 1) PRA increased from 55 ng angiotensin/100 ml in supine position to 120 ng/100 ml after fast rapid tilting. After a second, more prolonged tilting PRA was 110 ng/100 ml.

In case 2 (Fig. 2) PRA increased from 125 ng/100 ml in supine position to 175 ng/100 ml after tilting.

In case 3 (Fig. 3) PRA was 130 ng/100 ml in supine position before tilting. During the 60 min tilting period PRA was, after 30 and 60 min, 80 ng/100 ml and 30 ng/100 ml respectively.

PRA was before the noradrenaline infusion (Fig. 4) 45 ng/100 ml, at the end 25 ng/100 ml, and 20 min after the end of infusion 45 ng/100 ml.

DISCUSSION

The tilting procedure in the three patients with postural hypotension induced a varying response in PRA. Two of the subjects reacted with only a minor increase and one with a fall in PRA. There are several possible explanations of this. The three subjects differed somewhat with regard to cardiovascular function. In all of them there was a promp fall in blood pressure on head-up tilting. The two subjects who reacted with a minor increase in PRA also showed a slight increase in heart rate during tilting. The third subject who reacted with a decrease in PRA did not increase in heart rate during tilting. This subject probably represented the most complete type of postural hypotension. The possibility of different types of postural hypotension has been discussed in greater detail previously (13). Furthermore, blood flow and blood distribution may influence renin release and the possible changes in renal blood flow secondary to the low blood pressure during head-up tilting in these subjects cannot be evaluated. The influence of the decreased renal function in one of the subjects (no. 1), as indicated by the elevated serum creatinine and the concomitant hypertension in another (no. 2), are also impossible to evaluate.

Our results agree with the results of Gordon et al. (9) who used a somewhat different technique. They observed no change in PRA in patient with postural hypotension when the orthostatic stress was induced by walking during a period which was of longer duration than the head-up tilting in this study.

The results of Lewis et al. (12) may appear contradictory. They reported that three patients

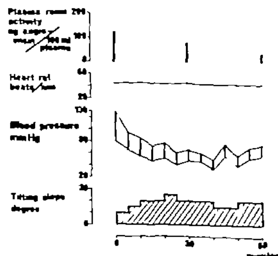


Fig. 3. Effect of tilting on heart rate, blood pressure and PRA in case 3.

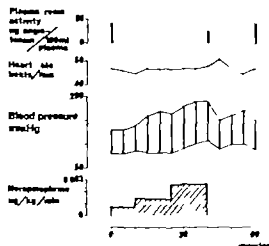


Fig. 4. Effect of noradrenaline infusion on heart rate, blood pressure and PRA in case 3.

with transplanted functionally denervated kidneys retained their normal renin response to upright posture. The possibility that the renal nerves play a role in the intact normal kidney can, however not be excluded from their studies. Gordon et al. (9) found that low doses of noradrenaline and adrenaline, 10 1 given to their patient with postural hypotension produced an increase in PRA. It is therefore possible that the juxtaglomerular cells in the transplanted kidneys (17) exhibited denervation hypersensitivity and reacted to the catecholamines released from the adrenal glands and/or extra-renal sympathetic nerve endings.

In the present study noradrenaline was infused into the patient with the most complete postural hypotension in a dose which caused a significant blood pressure rise, but PRA did not change. Noradrenaline may therefore be less potent than adrenaline in releasing renin in vivo. This discrepancy may be secondary due to different cardiovascular response to noradrenaline and adrenaline in vivo or to a different direct effect on the receptors responsible for the renin release. Božović and Castenfors (5) found that the renin release induced by hemorrhagic hypotension in rats was inhibited by the beta-receptor blocking agent Inderal®. Assaykeen et al. (1) showed that the renin release induced by hypoglycemia in was also prevented by Inderal®. These findings suggest that a beta-receptor mechanism is involved in renin release, but further studies are needed to elucidate the exact mechanism.

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VALIDITY OF BLOOD PRESSURE MEASUREMENT WITH CUFF IN THE ARM AND FOREARM

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Abstract. The arterial blood pressure has been measured with cuff in the arm and forearm of 44 patients, and the cuff pressures were compared with contralateral, intraarterial brachial pressures in another 52 patients. 1) A technique for recording of Korotkoff sounds and pressures in the forearm with standard cuff is described. 2) There were no systematic differences between contralateral arms or forearms as regards intraarterial or cuff pressures. 3) The cuff systolic as well as diastolic pressure was higher in the forearm than in the arm. 4) Systolic intraarterial pressure agreed well with corresponding contralateral arm cuff pressure but intraarterial diastolic pressure was systematically lower than corresponding cuff pressure. 5) The absolute pressure level, arm circumference or skinfold thickness did not influence the results given under paragraphs 3 and 4 as long as the standard cuff was used. 6) In extremely obese patients necessitating the use of big cuff on the arm there was big difference between intraarterial and cuff systolic or diastolic pressure, but use of the standard cuff on the forearm gave good results.

The purpose of the study was to test the validity of the cuff method by comparing it with intraarterial measurements and to apply the cuff method to the forearm, the idea being that it should solve the problem of blood pressure measurement in obesity.

MATERIAL

A comparison was made of cuff measurements in the arms and forearms of 44 patients. In another group of 52 patients bilateral cuff measurements were followed by simultaneous recordings intrabrachially and with cuff in the contralateral arm and forearm. All patients had sinus rhythm. The mean age for all 96 patients was 52.6 years with range of 18–83 years.

The non-obese patient was defined as having right arm circumference of 31 cm or less. The hypertensive patients had right arm systolic cuff pressure of at least 160 mmHg or diastolic pressure of at least 100 mmHg. Among patients with right arm circumference of 41 cm or more the standard cuff had to be replaced by the big cuff ordinarily used for the thigh, the idea being that such cuffs are usually reliable. These patients constitute the "big cuff" group in contrast to the "standard cuff" group. The forearm measurements were made with the standard cuff in this group.

Descriptive data on weight, height, arm circumference, skinfold thickness and arm systolic and diastolic cuff pressures from the 96 patients are given in Table I.

METHODS

The right arm circumference was measured at the maximal point. Skinfold thickness was measured over the left triceps with Harpender caliper.

All patients were investigated in supine position, with their arms abducted about 45° and the brachial artery approximately 5 cm dorsal to the sternal angle. This point was also taken as the zero reference level during intraarterial pressure recordings. The forearm was on the same level as the arm.

The standard cuff measured 12 × 41 cm, and the rubber bladder 11.5 × 29 cm. It was connected to mercury manometer (Erkameter, Germany). The big cuff was 12 × 84 cm, and the bladder 11.5 × 61 cm.

Cuff measurements were alternatively started on the right and left side and also alternatively on the arm and forearm. Systolic and diastolic pressures were recorded according to the recommendations of the American Heart Association (5), implying that the disappearance of Korotkoff sound was taken as the diastolic point.

Auscultatory blood pressure measurements in the forearm have been reported to yield only 79% success (4). However, in our study it was possible, in all but one patient, to hear the Korotkoff sounds over the radial artery if the hand and forearm distal to the cuff were made bloodless before and during inflation of the cuff. This was accomplished by asking the patient to clench the fist and at the same time compress the forearm below the cuff.

A needle of the Cournand type was introduced into the left brachial artery and connected via polyethylene tube to the pressure transducer (EMT 34 Elema-Schönander). The pressure was recorded with direct writing amplifier (Ultralett ABEM, Sweden).

Table I *Descriptive data of the patients*

Intraarterial means intraarterial measurements made. *Only cuff* means only cuff measurements made. *Cuff pressures* in the intraarterial groups are those taken before catheterisation

	Right arm, circumference (cm)		Left arm, skin-fold thickness (mm)		Weight (kg)		Height (cm)		Right arm, systolic cuff pressure (mmHg)		Right arm, diastolic cuff pressure (mmHg)	
	Only cuff	Intra-arterial	Only cuff	Intra-arterial	Only cuff	Intra-arterial	Only cuff	Intra-arterial	Only cuff	Intr arterial	Only cuff	Intra-arterial
Obese hypertensive												
	6	21	2	19	6	21	6	21	6	20	6	20
$\bar{x}$	36	35	21 3	22.4	85.3	84.2	160.6	170	169	174	98	107
s	3.3	2.4	12.3	9.2	12.4	13.0	15.8	8.0	10.7	21 7	14 7	19.7
Obese normotensive												
	6	7	3	5	6	7	6	7	6	7	6	7
$\bar{x}$	35.5	34	20 7	18	87	84.[illegible]	173	174.6	133	141	76	86
s	2.2	2.7	6.3	7.2	12.3	7.8	13.1	6.5	10.3	9.8	17.2	5 6
Non-obese hypertensive												
	12	12	3	10	12	12	12	12	12	12	12	12
$\bar{x}$	27	29	12.6	20 2	62.7	67.6	163.8	167 1	180	202	99	113
s	3.2	1.6	5.3	9.0	11 6	10.6	7 1	6.7	22.7	27.8	4.0	15.9
Non-obese normotensive												
	19	7	4	6	19	7	19	7	19	7	19	7
$\bar{x}$	28	29	14	16	61.9	71.6	165 4	174 6	124	140	71	86
s	2 5	2.0	4 1	9 8	9.8	9.3	9 7	11.6	13.9	9.6	11.9	5.3
Big cuff" group (extremely obese)												
	1	5	—	5	1	5	1	5	1	5	1	5
$\bar{x}$	41	46	—	36	96.5	122.7	163	162	180	210	110	112
s	—	4.5	—	3.1	—	[illegible]3 1	—	3 9	—	44.3	—	32.1

The pressure recording system (Fig. 1) was calibrated against four mercury manometer pressures, the highest about 20% above the actual systolic pressure. The zero level on the glass tube of the mercury manometer was fixed, but as the inner diameters of the two communicating vessels have a ratio of 1 12, the error from fixed zero amounts to only 1.5–2 0 mm of mercury at calibration pressure of 250 mm, and less at lower calibration pressures. The total system from needle to recorder was also dynamically tested. In 12 tests on 12 different occasions the degree of damping was 0.13–0.26 with a mean of 0.19 and the undamped natural frequency was 19–62 with mean of 31 Graphically estimated, the driving frequency at which the amplitude response was 1.02 varied from 3 to 9 with mean of 5

During continuous intraarterial pressure recording with a paper speed of 25 mm sec the cuff measurements were made on the opposite arm and forearm. The appearance and disappearance of the Korotkoff sounds were signalled as electrical markings on the recording paper The reference pressure started and ended the registration.

The cuff reading did not affect the intraarterial pressure. The average systolic and diastolic pressures of at least 10 cycles were calculated between the two markings for systolic and diastolic cuff pressures measured simultaneously

The analysis of results is based on computer calculations and visual inspection of graphs. Tests of significances were made on a 5% level.

RESULTS

Comparison between cuff pressures on right and left side

Included were all patients in Table I except one in whom the forearm pressure was not recorded

There was no significant difference between the right and left systolic or diastolic cuff pressures in either arm (systolic difference $n=95$ mean difference 1 mmHg standard error 1 1 diastolic difference, $n=95$ mean difference 0 mmHg standard error 0.9) or forearm (systolic difference, $n=95$ mean difference 2 mmHg standard error 1 0 diastolic difference $n=94$ mean difference 1 mmHg standard error 1.0). Hypertensives did not differ from normotensives, nor obese from non-obese. The distribution of points in the four bivariate comparisons were all rectilinear with a spread around the regression line unrelated to the absolute pressure level.

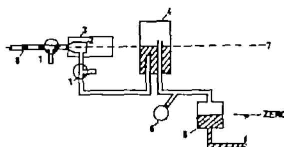


Fig. 1 Schematic drawing of the arrangement for pressure registration. Not to scale. *1* three-way stopcock, pressure chamber *3* pressure transducer *4* saline bottle *5* mercury manometer *6*, balloon for inflation; *7* level five cm below the sternal notch *8*, catheter to the patient.

Comparisons between right arm and forearm cuff pressures

Included were the same 95 patients as in the previous comparison. As the right and left side gave similar results, only measurements on the right side are presented.

From plots of differences between arm and forearm pressures versus arm circumferences it was clear that the "big cuff" group differed from the standard cuff" group. Consequently the two groups were treated separately In the standard cuff" group there was no evident correlation of systolic or diastolic difference to arm circumference, nor did skinfold thickness influence the arm-forearm pressure relationship.

Our standard cuff" measures systolic pressure significantly higher in the forearm than in the arm ($n=89$ mean difference arm–forearm -7 mmHg; standard error 1.4) Normotensives did not differ from hypertensives, nor obese from non-obese. The diastolic pressures behaved similarly but the absolute as well as the relative difference is bigger than for systolic pressure ($n=88$ mean difference arm–forearm -11 mmHg standard error 0.9)

In the "big cuff" group the results are reversed, thus systolic and diastolic pressures are higher in the arm than in the forearm (systolic difference, $n=6$ mean difference arm–forearm 22 mmHg; standard error 8.2 diastolic difference, $n=6$ mean difference arm–forearm 11 mmHg standard error 3.2).

Comparison between intraarterial and cuff pressures (Table II)

In the standard cuff" group there is a close average agreement between arm systolic pressure measured with cuff and intraarterially the former being 4 mmHg higher The forearm systolic pressure measured with cuff agrees slightly less well with intraarterial recordings, the former

Table II. *The relationship between brachial pressure and simultaneous contralateral indirect pressure*

P brachial pressure, *I* contralateral indirect pressure, *d* = difference *S* = individual standard deviation   correlation coefficient, *b* = regression coefficient. −significance of difference

Patient group	I		II		III		IV		I–IV		V	
Cuff position	Obese		Non-obese		Hypertensives		Normotensives				"Big cuff" group (extremely obese)	
	Arm	Forearm	Arm	Forearm	Arm	Forearm	Arm	Forearm	Arm	Forearm	Arm	Forearm
Systolic pressure												
	28	28	19	19	33	33	14	14	47	47	5	5
d	−3	−7	−6s	−8s	−5s	−8s	−3	−5	−4s	−7	−36s	−10
S	9.6	10.2	10.6	10.6	11.4	10.2	12.9	9.8	12.1	10.0	14.6	9.3
$S_{\bar{d}}$	1.8	1.9	2.4	2.4	2.0	1.8	3.5	2.6	1.8	1.4	6.6	4.2
	0.93	0.94	0.95	0.95	0.92	0.92	0.68	0.84	0.93	0.95	0.96	0.98
b_{PI}	1.05	0.97	0.92	0.98	0.97	0.89	0.77	0.99	0.99	0.97	1.08	0.97
S	0.08	0.07	0.07	0.08	0.07	0.07	0.24	0.18	0.06	0.05	0.17	0.11
Diastolic pressure												
d_{P-I}	−14s	−4	−14s	23s	−15s	−24s	−14s	−24s	−15s	−13s	−30s	−21s
S	11.1	8.5	6.4	5.0	9.9	8.1	8.3	5.3	9.7	7.8	10.0	8.3
$S_{\bar{d}}$	2.1	1.6	1.5	1.1	2.3	1.4	2.2	1.4	1.4	1.1	4.5	3.8
	0.91	0.93	0.92	0.96	0.92	0.93	0.76	0.90	0.91	0.93	0.95	0.96
b_{PI}	1.13	0.95	0.93	1.04	1.15	0.99	0.73	0.74	1.07	0.98	1.08	1.07
S	0.10	0.08	0.09	0.08	0.09	0.07	0.18	0.10	0.07	0.05	0.20	0.17

being 7 mmHg higher Normotensives, hyper tensives, obese and non-obese behave similarly

Arm diastolic pressure measured with cuff did not agree with intraarterial recordings, the former being significantly (15 mmHg) higher The dif ference is even larger for the forearm, 23 mmHg. Normotensives, hypertensives, non-obese and obese behave similarly

Arm circumference or skinfold thickness did not influence the indirectly-directly measured pressure relationship

In the big cuff" group arm systolic pressur measured with cuff deviates greatly from intra-arterial recordings, the former being 36 mm higher With the standard cuff" used in th forearm the difference is insignificant, 10 mmHg. Diastolic arm as well as forearm pressure measured with cuff is considerably above the intra arterial readings. Thus, in these patients, only the systolic forearm pressure measured with standard cuff agrees reasonably well with intraarterial recordings in the arm, and the individual standard deviation of the difference is similar to that in the standard cuff" group or around 10 mmHg.

DISCUSSION

onventionally intraarterial blood pressure is ven as the average systolic/diastolic pressure in a circulatory steady state. It is the relation between these pressures and pressures measured with the cuff method which is important to know Therefore we disagree with Berliner et al (3) who thought cuff pressures should be compared with the maximal intraarterial systolic and minimal diastolic pressures recorded in the same arm before or after the indirect measurement

Berliner et al. also wrote that quoting cuff pressure values with zero or five as the last digit shows that the personal factor is a major source of error We believe such quoting to be evidence of good judgement of measured quantities. Errors due to the preference for even numbers are a general experience which has also been demonstrated in cuff pressure recordings (2.) Besides that, a damping device in conventional mercurial manometers makes reading of pressure to the closest mm of mercury rather meaningless. This is empirically easy to test by looking at the variations for each heart cycle using an undamped aneroid manometer

With needle or catheter pointing against the blood stream and without side holes upstream, end pressures are recorded, which theoretically differ from the true lateral pressures. However according to Cyvin et al. (7), who measured with a needle, there is no difference between pressure recorded against the stream and the pressure recorded with the stream in the brachial artery of resting man. This agrees with the findings of van Bergen et al (2).

The findings of Shock and Ogden (24) and Simpson et al (25) support the belief that, with a rigorously standardized technique, the individual interobserver error within a group of investigators may be substantial but the systematic error is of the order of a few mm of mercury

London and London (18) have analysed the hemodynamic events peripheral to the cuff in relation to its compression and decompression and to the Korotkoff sounds. They found the peripheral average maximal and minimal pressures at the time of appearance and disappearance of Korotkoff sounds different from the reference levels, which were the average systolic and diastolic pressure in the same arm. The disagreement was particularly large for systolic pressure. These results may be due to factors such as the stenosing effect of the cuff peripheral blood filling and local neurogenic influence on the venal bed.

With a needle or catheter positioned close to the lower border of the cuff we found it sometimes difficult to hear the Korotkoff sounds. The needle or the catheter has a square area, which is a substantial part of the vessel area, and there is the risk that the presence of intraarterial measuring devices may influence the indirect method.

Our conclusion is that cuff pressures cannot be compared with simultaneous directly recorded pressures in the same arm at the same point.

Direct and indirect blood pressure measurements on the right and left side do not differ in our study This is in accordance with Harrison et al. (10). The explanation of differences found by others (10) should probably be looked for in the methodology

The arterial pulse wave undergoes successive changes during its peripheral propagation. According to Wood et al. (30) the intraarterial radial systolic pressure in normals is about 6 mmHg higher and the radial diastolic pressure about

Table III. *Comparisons between systolic direct and indirect pressure. Unless otherwise stated, each material consists of patients with normal as well as high pressure. If possible, the results have been estimated from figures or data*

Individual S.D = 1 S.D. from the average difference. Mean values within parentheses

Author	Year	No. of pats.	Average difference of pressures: Indirect direct difference ≤10 mmHg	Indirect < direct difference >10 mmHg	Indirect > direct difference >10 mmHg	Variability: Individual S.D	Corr coeff	Comments
			Comparison in the same arm					
Steele	1942	39		(10)				Comparison with radial artery. Hamilton manometer
Kotte et al.	1944	18						Hamilton manometer
Kotte et al.	1944	10						Hamilton manometer. All patients with aortic regurgitation
Roberts et al.	1953	30					0.89	Two different electro-manometers simultaneously giving different results
		47		(12)			0.89	
van Bergen et al.	1954	70						Biggest difference found. Increasing pressure—increasing difference. In the immediate post operative stage
Henschel et al.	1954	11				8.1		All normal young subjects. Comparison with radial artery
Godden et al.	1955	35				10.4		All normal young subjects. Comparison with radial artery
Trout et al.	1956	6			(46.5)			All subjects very fat
Berliner et al.	1960	100						Sixty-eight fat subjects. Comparison with maximal systolic pressure
Alexander et al.	1962	16						All subjects very fat
Karvonen et al.	1964	53				12.3		In the immediate post-operative stage
King	1967	25					0.31	Comparison with brachial or radial artery
			Comparison in contralateral arms					
von Bonsdorff	1932	25						Bröemser's glass plate manometer
Ragan et al.	1941	40						
Ragan et al.	1941	11						All patients with aortic insufficiency
Imhoff et al.	1958	40						Normotensives: no mean difference. Hypertensives = indirect direct pressure
Holland et al.	1964	47		(24.6)		14.0	0.95	
Simpson et al.	1965	24				10.4		
Karlefors et al.	1966	38				8.6	0.98	
Present study		47				12.1	0.93	
Present study		5			(36.0)	14.6	0.96	All patients very fat. Big cuff

Table IV *Comparisons between diastolic direct and indirect pressure Unless otherwise stated, each material consists of patients with normal as well as high pressure When possible the results have been estimated from figures or data*

Individual S.D 1 S D from the average difference
Roman figures indicate the sound phase at which the diastolic cuff pressure was recorded

			Average difference of pressures					
			Indirect ≈ direct difference ≤ 10 mmHg	Indirect < direct difference > 10 mmHg	Indirect > direct difference > 10 mmHg	Variability		
Author	Year	No. of pats				Individual S D	Corr coeff	Comments
			Comparison in the same arm					
Steele	1942	39						Comparison with radial artery (V)
Kotte et al.	1944	18						Hamilton manometer (IV)
Kotte et al.	1944	10						Hamilton manometer All patients with aortic regurgitation (IV)
Roberts et al.	1953	30					0.89	Two different el. manometers
Roberts et al.	1953	47					0.88	Similar result with both (V)
Bergen et al	1954	70						Biggest difference found. Increasing pressure—increasing difference. In the immediate post-operative stage (V)
Henschel et al.	1954	11				5 8		All normal young subjects. Comparison with radial artery (V)
Godden et al.	1955	35				8.2		All normal young subjects. Comparison with radial artery (V)
Trout et al.	1956	6			(36.0)			All subjects very fat (IV or V)
Berliner et al	1960	100						Sixty-one fat subjects. Comparison with intramural systolic pressure (V)
Alexander et al	196.	16						All subjects very fat (IV or V)
Karvonen et al	1964	53				14.4		In the immediate post-operative stage. Comparison with radial artery (V)
King	1967	29			(12.6)		0.56	Comparison with brachial or radial artery (V)
			Comparison in contralateral arms					
von Bonsdorff	1932	25						Brömser glass plate manometer (V)
Ragan et al.	1941	40						(IV)
Ragan et al.	1941	11						All patients with aortic regurgitation (IV)
Imhof et al.	1958	40						Diastolic pressure at appearance of sound at cuff compression
Holland et al.	1964	47		(13 1)		9.5	0.93	(V)
Simpson et al.	1965	23				10 4		(V)
Karlefors et al.	1966	18			(11 3)	9 9		(V)
Present study		47			(15 0)	9 7	0.91	(V)
Present study		5			(30 0)	10 0	0 95	All patients very fat Big cuff (V)

mmHg lower than the brachial pressure. These differences were somewhat higher in hypertensives.

Our finding that the forearm systolic cuff pressure is on an average 7 mm of mercury higher than the intraarterial brachial pressure indicates a close agreement between direct and indirect systolic forearm pressure. Our diastolic forearm cuff pressures were on an average 23 mm higher than the brachial pressure, indicating that the cuff overestimates the intraarterial radial pressure by approximately the same amount.

We found the standard cuff" measurement of systolic pressure to be 7 mm higher in the forearm than in the arm. Diastolic pressure was 11 mm higher in the forearm. Rytand and Boyer (23) found the systolic pressure 6 mm lower and the diastolic pressure 6 mm higher in the forearm. Blackburn et al. (4) found no difference between the arm and forearm systolic pressure, but the indirect diastolic pressure was about 4 mm higher in the forearm. They as we, found arm circumference and skinfold thickness to be without influence on the arm-forearm cuff pressure difference.

It may be concluded that the exact arm-forearm relationship with regard to indirect blood pressure measurements has not been clarified, but it seems that the standard cuff applied to the forearm gives systolic values in good agreement with arm pressures and with the intraarterial pressures in the forearm, which deviate little from intrabrachial values.

Blackburn et al (4) could not see any advantages using forearm cuff measurements. Our data on non-obese and moderately obese patients agree with their opinion. On the other hand we found, like others (8, 29), that the validity of the cuff method increases in extreme obesity by using standard cuff on the forearm. By a special technique for cuff inflation, we have been able to make the Korotkoff sound well udible, which believe is practically important For scientific purposes it may be possible to get better results in extremely obese patients by use of cuff somewhat smaller than our big cuff In practical work the thigh cuff is mostly the only available alternative, which, however should be replaced by the standard cuff applied to the forearm Our comparison between direct and indirect measurement in the arm should be interpreted in relation to other studies, which have been collected in Tables IV and V Only studies using a cuff with a bladder size close to 12 × 25–30 cm have been tabulated unless otherwise stated. Variations in cuff width of about 12 ± 2 cm do not seem to have any substantial influence on the results (26).

The comparisons of systolic direct and indirect pressures in Table III do not show any characteristic difference between investigations in the same arm and in contralateral arms. It seems to be most common that cuff and intraarterial pressures are on an average roughly the same, and that the standard deviation of the difference is around 10–15 mmHg. This was also our own finding.

The comparisons of diastolic direct and indirect pressures in Table IV differ In studies in the same arm the majority show cuff pressures approximately the same as intraarterial pressures, and the standard deviation from the mean difference is around 5–15 mmHg. Among studies with contralateral arm cuff pressures tend to be equal to or as in our study higher than intraarterial pressures, and the standard deviation from the mean difference is around 10 mmHg.

It may be questioned whether studies made in the immediate postoperative stage should be compared with studies made on patients in hemodynamic balance. However the two studies made in that way (2, 15) show contradictory results.

The two studies of patients with aortic regurgitation (17 21) show different results with regard to diastolic pressure.

We did not see the error of the cuff vary with the absolute pressure level, as found by van Bergen et al. (2) and by Imhoff and Hürlimann (13).

Th. influence of obesity on cuff readings is controversial The two studies with very fat patients are contradictory (1 29). The general opinion is that the cuff tends to overestimate systolic and diastolic pressures. The study of Alexander et al (1) is, however an exception.

As regards moderate obesity Ragan and Bordley (21) concluded that arm circumference affects the error of cuff measurements. Their opinion had a great impact, probably due partly to the prominent place it received in Pickering book on hypertension (19). Pickering et al. (20) in f ct worked out tabl for correction of cuff pressures at different arm circumferences.

Others have presented data in favour of a minor influence of arm circumference on the error or even no effect of practical importance (3 12, 15 16). Our results are in accordance with this latter view

The presence of a positive correlation between obesity and hypertension has been sometimes questioned and explained by an overestimation of cuff pressures in obese patients. However Tibblin (28), in his population study of men born in 1913 found a clear positive correlation between obesity and high cuff pressures and also other hypertensive manifestations. This is a further point in favour of th. opinion that cuff values are as valid in obese as in non-obese patients.

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HEREDITY IN PRIMARY HYPER-β-LIPOPROTEINEMIA WITH CONCOMITANT XANTHOMATA

Report on Three Kindreds with Presumably Six Homozygotes

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Abstract. Three families with frequent occurrence of hyper-β-lipoproteinemia, xanthomatosis and ASCVD have been analysed. Khachadurian's hypothesis, that in families with primary familial hypercholesterolemia the homozygotes are affected earlier and more severely than the heterozygotes, is tested and confirmed. The distribution in the families of cases with early and severe hypercholesterolemia, extensive xanthomatosis, and early cardiac death is in accordance with the thesis that primary hyper-β-lipoproteinemia, which formerly was defined as familial hypercholesterolemia, has a dominant mode of heredity with incomplete penetrance and variable expressivity.

Since 1933 one of us (M. K. P.) has followed two kindreds with famillial hypercholesterolemia. In 1964 a third family became known. We give briefly the case histories, taking into account that primary hypercholesterolemia is nowadays identified with primary hyper-β-lipoproteinemia. For the classification of the different types of hyperlipoproteinemia we refer to the publications of Fredrickson and Lees (4), Fredrickson et al (5), and of our own group (2, 3, 12, 13, 14).

After 1961 full estimations of the various lipid and lipoprotein fractions were made in the surviving members of these kindreds, which proved that their hypercholesterolemia was in fact caused by hyper-β-lipoproteinemia. The earlier cholesterol and phospholipid determinations proved to be comparable within a 10% limit with those estimated by the methods used more recently (14).

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CASE REPORTS

Kindred A

In 1933 a girl with xanthomata and hyperlipidemia was observed (A III, 1). A brother (A III, 2) and two cousins (A III, 9 and 10) had the same disorder. These four children were the offspring of two brothers (A II, 1 and 3) who had married two sisters (A II, 2 and 4). The four affected children (A III, 1, 2, 9 and 10) all died suddenly before the age of 17, presumably from a cardiovascular accident. This kindred has been described previously (6, 10, 11).

In 1966 we were able to re-examine this family and include an investigation of the lipoprotein contents of the sera of the three still living parents (A II, 1–3) of the affected children. A II, 1 and 2, both had a hyper-β-lipoproteinemia (Table I) and both suffered from angina pectoris, complicated by myocardial infarction, which was confirmed by ECG studies. Their two surviving children, A III, 3 and 5, had no clinical symptoms according to their family physician. Blood studies could not be carried out.

Of the other couple (A II, 3 and 4) only the husband (A II, 3) was still living. He had a history of coronary infarction after the age of 50, but the blood-lipid values were repeatedly normal. His wife had died from uremia at the age of 64; no apparent heart complaints had been present.

In 1932 Jordans and van der Horst (6) estimated in both couples the cholesterol content of the blood, which was already elevated in A II, 1 and 2 (394 and 344 mg%), the other couple (A II, 3 and 4) showing normal values (174 and 218 mg%).

The data on other relatives are given in Table I, but for our purpose only the parents, grandparents and offspring of presumable homozygotes are important.

Kindred B

In 1933 a 4-year-old boy (B II, 1) with extensive xanthomatosis (Fig. 3), hypercholesterolemia (1 066 mg%) and hyperlipidemia was examined.

Table I. *Lipid and lipoprotein values of members of three kindreds with primary hyper β-lipoproteinemia*

The reference group comprises 25 healthy persons (mostly students and physicians) with median ge of 27 years, ranging from 6 to 55 years, and roughly equal representation of the sexes
+ = symptom present, 0 symptom bsent, — = unknown, *x* = xanthelasma palpebrarum, *x* = xanthomata.
ASCVD arterio-sclerotic ascular disease

			Sex	Total lipids (mg 100 ml)	Total cholesterol (mg 100 ml)	Lipids from lipoproteins (mg 100 ml)	Lipids from β-lipoproteins (of total lipid)	*x*	*x*	ASCVD	Cardiac death at age	Living at age
Presumed homozygotes												
A	III	1		—	1 080	—	—	0	+	+	13	
	III		♂	—	836	—	—	0	+	+	15	
	III	9	♂	—	692	—	—	0	+	+	16	
	III	10	♂	—	647	—	—	0	+	+	16	
B	II	1	♂	—	1 066	—	—	+	+	+	30	
C	III	2	♂	2 634	1 177	2 450	93	0		0		2
Presumed heterozygotes												
A	II	1	♂	1 163	388	768	66	0	0	+		63
	II	2		1 413	512	1 116	79	0	0	+		64
	II	3	♂	651	196	391	60	0	0	+		72
	III	7	♂	1 011	360	667	66	0	0	0		52
	III	11		953	297	534	56	0		0		41
B	I	1	♂	—	403	—	—	+	0	+	44	
	I	2		1 388	334	1 055	76	0		+		60
	III	1	♂	944	354	699	74	0	0	0		10
	III	2	♂	841	284	555	66	0	0	0		8
C	I	2		1 467	527	998	68		+	+		54
	I	3	♂	1 353	461	879	65	0	0	+		65
	II	3		1 308	534	1 073	82	0	0	0		21
	II	6 (in 1967)	♂	758	251	485	64	0	0	0		22
	II	6 (in 1969)		846	311	590	65	0	0	0		24
Presumed normal												
	II	2		576	185	334	58	0	0	0		34
	II	3	♂	760	223	403	53	0	0	0		38
	II	4		792	263	523	66	0	0	0		33
	II	5	♂	914	278	457	50	0	0	0		23
	III	3		597	184	316	53	0	0	0		13
	III	4	♂	606	199	303	50	0	0	0		4
C	I	4		834	245	433	52	0	0	0		64
Reference group range				542–934	154–286	220–506	35–63					

This patient was observed regularly until his death During this period the tuberous xanthomata grew in size, palpebral xanthelasmata ppeared at the age f 12, the first cardiac complaints occurred at the age of 10, and angina pectoris and signs of ischaemia in the ECG were observed at the age of 13 Death from cardiac failure followed at the ge of 30 years

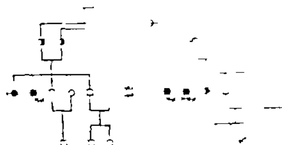


Fig 1 Pedigree A.

Male Female
Not investigated, anamnestically normal
Individual xamined clinically and biochemically all esult ithin normal limits
Presumed heterozygote, biochemically and clinically investigated all esult utside normal limit
Presumed heterozygote, ascertained by estimation of blood cholesterol and of β lipoprotein content
Presumed homozygote (full xamined)
ASCVD (arteriosclerotic oronary ascular disease)
Tuberous and or tendinous xanthomata
Cardiac death.

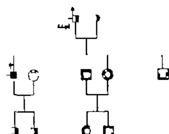


Fig. 2. Pedigree B. Symbols as in Fig. 1. B I, 1 and B II, 1 showed also xanthelasmata palpebralia.

In the father of this boy (B I, 1) we found hyper cholesterolemia (403 mg %) at the age of 27 palpebral xanthelasmata at 34, and the age of 40 the first complaints of angina pectoris appeared. H died at the age of 44 of coronary thrombosis.

At the age of 26 years the mother (B I, 2) had an elevated cholesterol content of the blood (334 mg%). Later angina pectoris developed. I 1967 detailed study of the lipoproteins showed the elevated cholesterol content to be due to hyper-β-lipoproteinemia (Table I). At that time she showed tendinous xanthomata, and the ECG showed signs of coronary sclerosis.

The proposita married normal woman (B II, 2) who was examined at the same time as their offspring in 1967. The children (B III, 1 and 2) were clinically normal, but both had an undeniable hyper-β-lipoproteinemia. The cholesterol content was, however much lower then that of the father at the same age. No skin or cardiovascular symptoms were present in the children.

Kindred C

A 1 ½-year-old boy (C III, 2) was seen for the first time with xanthomata of the skin folds resembling planar xanthomata (Fig. 5). The creases of the palms were unaffected. The clinical picture closely resembled that of the propositus of kindred B. Also the same excessive hypercholesterolemia (1 127 mg%) was found. Further analyses demonstrated hyper β-lipoproteinemia. The mother (C II, 3), the maternal grandmother (C I, 2), and the paternal grandfather (C I, 3) all had hyper-β-lipoproteinemia, the maternal grandmother had also palpebral xanthelasmata and tendinous xanthomata. The paternal grandmother (C I, 4) showed no blood abnormalities. The father of the boy (C II, 6) showed in 1967 β-lipid and cholesterol content just below the upper normal limits, but in 1969 these were distinctly elevated for his age (Table I).

During the observation period of 2 years the lesions in the propositus increased in size and number and typical tuberous xanthomata developed (Figs. 6 and 7).



Fig. 3. Tuberous xanthomata in presumable homozygous patient with hyper-β-lipoproteinemia. B II 1 at age 4 years.

DISCUSSION

Xanthomatosis has been considered for more than a century as an inherited disease (1 15). Later the underlying metabolic disorder (hypercholesterolemia, subsequently recognized as a manifestation of hyper-β-lipoproteinemia) was regarded as the genetic defect. Controversy has arisen on possible clinical and chemical differences between homo- and heterozygotes with this genetic abnormality. It was proposed that heterozygotes show only hypercholesterolemia, homozygotes xanthomatosis in addition (16). Other authors (8, 9) advanced the hypothesis that the presence of xanthomata and/or cardiovascular lesions is dependent on the time of exposure to and the degree of elevation of the cholesterol content of the blood. Finally it was proposed that in familial hypercholesterolemia the double dose of the gene causes earlier and higher elevation of the cholesterol content of the blood, more extensive xanthomatosis and more serious cardiovascular involvement than the single dose (7). We tested this hypothesis on our kindreds, in which the hyper

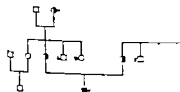


Fig. 4. Pedigree C. Symbols as in Fig. 1. C I, 2 showed also xanthelasmata palpebralia.



Fig. 5. Xanthomata resembling planar xanthomata in a very young (18-month-old) patient with presumable homozygous hyper-β-lipoproteinemia (C III 2).

cholesterolemia was proven to be due to an hyper-β-lipoproteinemia.

In Table I the investigated individuals are arranged according to their presumable genotype. We presumed that a patient would be a homozygote when a very high cholesterol content of the blood was combined with extensive xanthomatosis and early cardiac death. The parents and offspring of such patients should necessarily both be heterozygotes.

It is obvious from Table I that a clearcut distinction without any overlap exists between presumed homozygotes and heterozygotes. In the homozygotes there is a preponderance of males: five males as against one female. Within the group of homozygotes patient B II 1 is rather unusual since he reached the age of 30 and had two children. Both his parents and both his children evidently fulfilled the criteria for heterozygosity as required genetically if B II, 1 is to be homozygote.

The presumed heterozygotes show a somewhat wider range of expressivity while even the penetrance of the gene is not complete, since one of them (A II 3), who ought to be a heterozygote, shows no clinical or biochemical evidence of being so (with the exception of the presence of arteriosclerotic coronary vascular disease (ASCVD)). C II 6, who was assumed to be a gene carrier in 1967 proved in 1969 to be affected.

Family A seems to be the most unusual of this series. In both marriages of two brothers with two sisters, two severely affected presumably homozygous offspring occur. The parents A II, 1 and 2 are moderately affected, and are therefore presumably heterozygotes. A II 3 and 4, who also must be heterozygotes, are anamnestically normal. Only the blood of A II 3 could be studied, giving results within normal limits. We have no evidence of consanguinity between any of the grandparents in this pedigree.

According to the data in Table I in the presumed heterozygotes hyper-β-lipoproteinemia is present in a high proportion of the cases (11 out of 13) later in life often combined with palpebral xanthelasmata and in many cases also with tuberous and tendinous xanthomata. The incidence of ASCVD is much higher than could be expected in a group of individuals of comparable age. ASCVD may occur in patients with or without skin manifestations.



6



7

Figs. 6 and 7. Same patient as in Fig. 5. The lesions developed into typical tuberous xanthomata.

No factors favoring the manifestations of the gene could be demonstrated, although it is conceivable that diet and other habits favoring hypercholesterolemia play a role.

Our data consequently are in accordance with the hypothesis that primary hyper-β-lipoproteinemia is a familial disorder having a dominant mode of heredity with incomplete penetrance (1 out of 13 gene carriers having no manifestations) and variable expressivity

The homozygote shows a very high β-lipoprotein bloodlevel at or shortly after birth, which causes hypercholesterolemia and a moderate elevation of the phospholipid level. Expressed as a percentage of the total lipids, the sterols are elevated, and the triglycerid and phospholipid levels are proportionally low (14). When xanthomata appear during the first few months of life, they are first seen in skinfolds, and may be confused with the planar xanthomata (Fig. 2) occurring in primary hyperpre-β-lipoproteinemia (13). Unlike the planar xanthomata of the latter disease, they do not occur on the palms. Later in life (Figs. 6 and 7) tendinous and tuberous xanthomata appear in the homozygotes. Cardiovascular complications occur early Both coronary thrombosis and xanthomatous growth on the aortic valves are found at autopsy In our material four patients died between the ages of 13 and 16 and one at the age of 30 A boy aged 3 is still living.

ACKNOWLEDGEMENT

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SALICYLATE INDUCED HYPOPROTHROMBINEMIA

A Report of Four Cases

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Abstract. Four cases of severe hypoprothrombinemia caused by acetylsalicylic acid in therapeutic doses are reported. All cases were verified by positive provocation tests. The hypoprothrombinemia was accompanied by bleeding episodes in two of the four patients. Repeated salicylate medication constantly induced a lowering of the prothrombin-proconvertin level, but in spite of increased doses of salicylates the PP did not reach such low levels as those recorded initially. This observation, together with the findings of an incomplete normalization of the PP in two instances, implies that the hypoprothrombinemic effect of salicylates may be influenced by additional factors. Three patients had previously been subjected to partial gastrectomy and showed signs of malnutrition and malabsorption.

As early as in 1891 Binz (3) pointed out that salicylates might provoke mucosal bleeding. Douthwaite and Lintott (6) suggested that acetylsalicylic acid may precipitate massive hemorrhage from the gastrointestinal tract. Occult fecal blood loss as well as manifest bleeding due to ingestion of salicylates has been demonstrated by Lange (12), and later by other authors (1 25 26).

The mechanism is still in dispute. Gastroscopy has revealed the presence of injection and hemorrhagic erosions of the mucosa around particles of aspirin (6, 16). The findings, however, that parenterally administered acetylsalicylic acid may cause occult blood in the stools suggest that some systemic hemostatic mechanism may be involved (8). Furthermore, excessive bleeding from various parts of the body provoked by salicylat therapy has been reported (7 15 17 24 28). In these cases factors other than gastric irritation seem to be in operation.

Link et al. (13) in 1941 demonstrated that acetylsalicylic acid caused a prolongation of the prothrombin time in rats fed a vitamin K-deficient diet. Such prolongation has later been demonstrated in man kept on ordinary diet (1-). Dicumarol potentiates the hypoprothrombinemic effect, while vitamin K counteracts it. The functional effect of oral anticoagulants and acetylsalicylic acid appears to be similar (1) and it is of interest to note that the drugs have also a structural similarity. Many investigators, however, conclude that, even when salicylates are given in massive doses, the clotting factors will almost never be depressed to such a level as to result in hemorrhage (4 5 19). On the other hand Wising (28) and Magid and Christensen (15) have shown that otherwise healthy individuals may develop hypoprothrombinemia and hemorrhagic diathesis during salicylate medication.

The following four cases illustrate that severe hypoprothrombinemia may develop secondary to therapeutic doses of salicylates in patients of poor nutritional status.

CASE REPORTS

Case 1

Female born 1886. In 1957 she was admitted to our department with fever, chest pain, and symptoms of longstanding cardiac insufficiency including hepato-splenomegaly. She was underweight, with history of malnutrition. Except for low total serum-protein (5.6 g/100 ml), other pertinent laboratory examinations showed normal values. Blood cultures were negative.

Course. A diagnosis of rheumatic fever seemed probable. In addition to streptomycin for few days, the patient received acetylsalicylic acid (Magnyl®) in daily doses of 4 g (Fig. 1). Ten days after commencement of salicylate therapy she had short episode of gastrointestinal bleeding, and two days later new melena occurred. The prothrombin-proconvertin value (PP), which initially was 50%, had dropped to below 5%, but increased to 38% following an injection of vitamin K

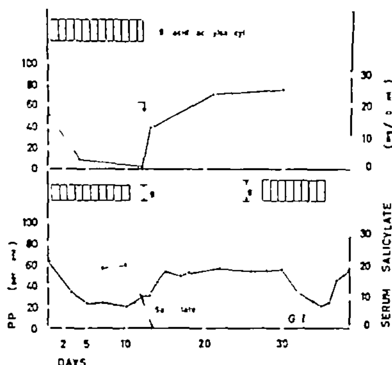


Fig 1 Case 1 — prothrombin-proconvertin values (PP) during acetylsalicylic acid medication. salicylate level.

(50 mg of menadion). After discontinuation f the salicylate therapy PP increased within a short time to normal range, and tests for blood in the stools became negative. A few eek later acetylsalicylic acid, 3 g w again given. PP this time fell to 1%, and m- slowly to about 50%, after withdrawal of the Some weeks later acetylsalicylic acid (4 g) was for third time, and again the PP level decreased t 1%. No further hemorrhage was observed. Normal values ere recorded for blood platelet count, bleeding time coagulation time and capillary resistance test. Moderate signs of cardiac insufficiency persisted throughout the investigation period. Daily injections of theophyllamin were given for many eeks.

Summary A patient showing symptoms of cardiac insufficiency with liver congestion, developed acetylsalicylic acid induced hypoprothrombinemia accompanied by gastrointestinal hemorrhag The PP was recorded at less than 5%. Repeated salicylate medication resulted in significant lowering of the PP

Case 2

Female born 1894. The patient had five years previously been subjected to partial gastrectomy (Moynihan II) (gastric ulcer). Post-operatively there was tendency to sideropenia with anemia. She was admitted to the hospital with symptoms of osteoporosis and anemia. She had postoperative weight loss of about 5 to 10 kg.

Laboratory findings. ESR 20 mm h, Hb 5.5 g/100 ml, MCH 19 μμg, MCV 83 c Serum-F 15 μg/100 ml, transferrin 500. There was practically no absorption of iron after an oral load, and excess f fat was found in the feces.

Course The general condition was gradually improving. Salicylate therapy (Astryl?), in daily doses of 6 g was given because of dorsalgia. The PP which was 90% prior to medication, dropped to 8% after three weeks of salicylate therapy and increased to 100 within one eek after withdrawal of the drug (Fig. 2). A provocation test, with the same salicylate dosage, rapidly induced hypoprothrombinemia. This time however the minimum PP was found to be 13%. Serum salicylate concentration varied between 35 and 40 mg/100 ml. During hospital stay for anemia one year later salicylate administration induced a decrease in PP to 56 th concomitant serum concentration between 20 and 40 mg 100 ml. This time the patient was generally in a fairly good condition.

Summary A partially gastrectomized patient, showing slight sign of malnutrition and malabsorption, developed reproducible hypoprothrombinemia during repeated salicylate medication.

Case 3

Female born 1902. In 1943 partial gastrectomy was performed (Moynihan II) (duodenal ulcer). In 1944 she suffered a massive hematuria hich led to hospitalization. The source of bleeding was not found. On admission the prothrombin time was markedly prolonged but became normal after injections of vitamin K. At the same time the hemorrhage stopped. There was no information of salicylate medication. While in hospital the patient developed jaundice and was opera d upon. The findings of dilated gall bladder and a pancreas hich was firm and swollen, led to diagnosis of chronic pancreatitis. A cholecysto-duodenostomy was performed. In 1950 she had an operation for ileus, and pancreas was then found to be normal on palpation. I Oct. 1954 she

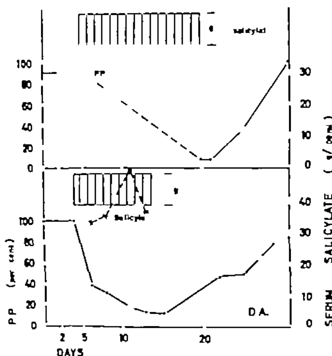


Fig. 2. Case 2. — prothrombin-proconvertin values (PP) during salicylate medication. — salicylate level.

developed painless hematuria, and on Nov 7 new episode of hematuria led to admission to this hospital. She was underweight, the postoperative loss of weight being about 10 kg. There were otherwise normal findings.

Laboratory findings. The PP was below 5%, ESR 42 mm/h, Hb 9.3 g/100 ml, MCH 25 µµg, MCV 85 µ. She had sideropenia, with defective iron absorption. Excessive fat and nitrogen content were demonstrated in the stools. Hepatoscintigraphy (Au^{198}) showed question-

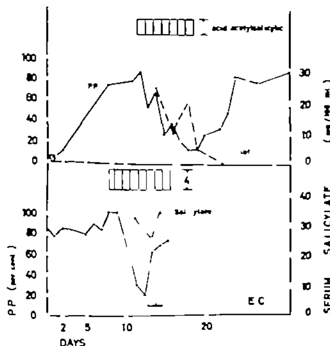


Fig. 3. Case 3. — prothrombin-proconvertin values (PP) during acetylsalicylic acid medication. salicylate level.

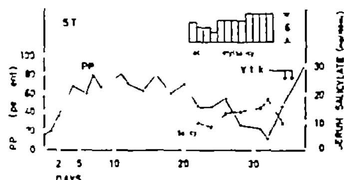


Fig. 4. Case 4 — prothrombin-proconvertin values (PP) during acetylsalicylic acid medication. -- salicylate level.

able incipient cirrhosis. The secretin test was not conclusive. Other pertinent tests were within normal limits. On the day of admission an urinary examination showed proteinuria and hematuria. The Gerhard's reaction was negative.

Course. The PP increased spontaneously and rapidly to normal and hematuria disappeared (Fig. 3). The source of bleeding could not be found. It could not be verified that the patient had taken salicylates prior to admission. Salicylate-induced hypoprothrombinemia was nevertheless thought to be the most probable cause. From Nov 17 she was given acetylsalicylic acid (Albyl Enterosolubile®), 3 g daily. On Nov 23 hematuria was noted, and on the following day PP was found to be 14%. The PP returned spontaneously to normal, but a new acetylsalicylic acid load of 4 g daily provoked a decrease of PP to 22% within three days. The serum salicylate concentration during the two trials with salicylate medication was about 20 and 30 mg/100 ml, respectively.

Summary. A patient who previously had undergone partial gastrectomy was hospitalized because of hematuria. She had a PP of less than 5%, which spontaneously returned to normal. Hypoprothrombinemia and hematuria developed during an acetylsalicylic acid load. A second provocation test induced a lesser decrease in PP in spite of higher salicylate dosage. The patient had steatorrhea, a highly doubtful liver cirrhosis and chronic pancreatitis.

Table I. *Factors that may have influenced the hypoprothrombinemic effect of salicylates*

	Case 1	Case	Case 3	Case 4
Partial gastrectomy				+
Malnutrition	(+)			
Malabsorption (steatorrhea)				
Chronic pancreatitis			?	
Liver disease	Congestion		Cirrhosis incip.	
Other drugs	Streptomycin (methyl-xanthines)			

Case 4

Male born in 1898. In 1949 he was subjected to partial gastrectomy (Moynihan II) (gastric ulcer). Symptoms of dumping and a tendency to sideropenia and anemia developed postoperatively and from 1962 there were signs of vitamin B_{12} deficiency. Because of chronic rheumatic polyarthritis (which was diagnosed in 1967) he had been treated with corticosteroids, and in the last two years with acetylsalicylic acid, 2 to 3 g daily. Because of dysphagia, bronchitis and malnutrition he was in March 1971 admitted to the Medical Department. His weight, which was now 39 kg, was 50 kg in 1967 and 60 kg prior to operation.

Laboratory findings. ESR 66 mm/h, Hb 12.2 g/100 ml. Serum-Fe was 39 and transferrin 164 µg/100 ml, total serum-protein 5.4 g/100 ml. There was slightly increased excretion of fat in the feces. On admission the PP was 16% and in urine a false positive Gerhard reaction was noted. Other tests were within normal limits.

Course. The patient gained 10 kg during the stay. The low PP value together with a history of acetylsalicylic acid medication, indicated a salicylate-induced hypoprothrombinemia. After withdrawal of the salicylate medication PP increased to more than 60% within two days but did not reach higher levels than 80% (Fig. 4). Three weeks later the therapy with acetylsalicylic acid was reinstituted. Owing to poor cooperation the daily salicylate dosage varied to some extent. When treatment had to be discontinued the PP had dropped to 13%. The PP increased to 100% after injections of 50 mg of vitamin K (menadion).

Summary. A patient who had undergone partial gastrectomy and who showed signs of malnutrition and malabsorption developed a reproducible salicylate-induced hypoprothrombinemia.

DISCUSSION

According to the literature salicylate induced hypoprothrombinemia of any significance, and especially as a cause of hemorrhagic diathesis, seems to be very rare. The effect of therapeutic doses of salicylates on vitamin K dependent coagula-

tion factors may however lead to manifest bleeding episodes in healthy individuals (15 28)

The four cases here reported were discovered more or less by chance, and their case histories indicate that certain categories of patients may be predisposed to salicylate-induced hypoprothrombinemia. The variable drop in PP on repeated salicylate medication, as well as incomplete normalization on withdrawal of the drug in two cases, implies that the effect of salicylates on PP may be influenced by other factors (Table I).

Our patients were in poor nutritional condition. Three of the patients had previously been subjected to partial gastrectomy It is not very likely that gastrectomy per se should result in a specific predisposition to salicylate-induced hypoprothrombinemia. On the other hand it is well known that anticoagulation-treated patients may show unexpected fluctuations in the prothrombin level, and this is probably caused by variable absorption of vitamin K. Malnutrition, partly as a result of postoperative dyspepsia and partly due to some degree of malabsorption, is a common sequela of gastrectomy (10).

Various hepatic diseases may potentiate the hypoprothrombinemic response to salicylates (2, 22). In one or possibly two of the reported cases, liver disorder may have been a contributing factor in provoking the hypoprothrombinemia.

Drug interaction is another point to consider Antibiotics and anticoagulants, as well as several other drugs (11), may potentiate the effect of salicylates, partly by a synergestic action on the synthesis of vitamin-K-sensitive coagulation factors. Concerning our first patient, it is of some interest to note that parenteral administration of methylxanthins has been claimed to reduce the prothrombin level (23).

It seems evident that salicylates may have a profound effect on the hemostatic mechanism. Recently it has been observed that aspirin in small doses prolongs the bleeding time in normal subjects, without affecting the prothrombin level (20) This impairment of hemostasis is probably due to an inhibitory effect on platelet function (9 27) Evidence has been produced showing that these drugs may increase capillary fragility (7) and cause thrombocytopenia (18), which secondarily may contribute to a bleeding tendency in som patients.

The present observation calls attention to the fact that salicylate therapy may inudce hypoprothrombinemia to such an extent as to cause hemorrhagic diathesis. The findings imply that certain groups of patients may be liable to this complication. The fact that salicylates exert both local and systemic effects on the hemostatic mechanism is of special clinical importance, considering the huge consumption of such drugs.

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DEPRESSION OF SYNOVIAL FLUID COMPLEMENT ACTIVITY AND RHEUMATOID FACTOR POSITIVITY

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Abstract. The total complement activity of synovial fluid has been determined in 215 arthritic patients, half of them were rheumatoid factor positive. Forty-[illegible] per cent showed rheumatoid factor negativity and normal complement values of the synovial fluid, and 45% showed rheumatoid factor positivity and depressed complement values.

The levels of the total complement (C) and the first four reacting C components (C1, C4, C2 and C3) of synovial fluid have been shown to be lower in rheumatoid arthritis (RA) than in various non-rheumatoid forms of arthritis (see review in ref. 9). Moreover, in recent studies, conversion of C3 was found to be more pronounced in rheumatoid than in non-rheumatoid synovial fluid (6, 15). These results are compatible with an activation of the C system in the rheumatoid joint.

In a previous study (4) it was shown that the synovial fluid C activity varied inversely with the rheumatoid factor (RF) titres in serum or synovial fluid. There was, however, an appreciable proportion of the RF positive RA patients in whom the synovial fluid C activity was not depressed according to the criterion used in that study. Using a more appropriate definition of depression in the present survey of the results obtained in 215 arthritic patients, it was observed that a depression of the synovial fluid C activity occurred in RA as often as RF positivity. Indeed, in RF positive RA it occurred in 88% of the patients.

MATERIAL AND METHODS

The clinical material consisted of 215 patients selected and classified as described elsewhere (4, 5), except that the term psoriatic arthropathy was restricted to polyarthritis involving the distal interphalangeal joints, polyarthritis involving mainly the large joints was labelled "atypical polyarthritis" (1, 7). Juvenile and adult forms of arthritis closely agreed in synovial fluid C activity, for which reason they were considered together (5). Only non-rheumatoid patients (see Table I) with negative sensitized sheep cell test were studied.

Concerning the statistical methods, and the techniques used for the determinations of the relative synovial fluid C activity and the RF titres, reference is made to a previous report (4). The relative synovial fluid C activity is defined as the synovial fluid-serum ratio for CH$_{50}$ units divided by the protein concentration of synovial fluid in g/100 μl. The activity was determined with a coefficient of variation of about 10% (4). Three separate RF tests were made: the sensitized sheep cell (SSC$_s$) test on whole serum, the latex test on the euglobulin fraction of serum (latex$_s$) and on that of synovial fluid (latex$_{sf}$). The latex$_{sf}$ and latex$_s$ were generally agreed, though occasional exceptions were noted.

The patients referred to as RF negative were negative by all three RF tests made, whereas of the RF positive patients RF positivity was demonstrated by three tests in 72.5%, by two tests in a further 20.2% (generally the latex tests), and by one test in only 7.3%. SSC$_s$ titres ≥ 32 and latex titres ≥ 20 were considered positive.

RESULTS

A. *The C activity of synovial fluid in non-rheumatoid arthritides*

In Table I four groups of various non-rheumatoid forms of arthritis are listed together with one group of miscellaneous non-rheumatoid arthritides. The average synovial fluid C activities in these five groups were very similar and statistically indistinguishable from each other. Therefore the five groups were pooled to form a group of 70 cases in which the C values showed a normal distribution around a mean of 99 (median 99.0, S.D. 22.0). This group is used as the reference

Table I *The complement (C) activity of synovial fluid in various forms of inflammatory arthropathies other than rheumatoid arthritis, SLE and SLE-like syndromes*

	Ankylosing spondylitis	Reiter disease	Oligo-arthritis	Psoriatic rthropathy	Miscellaneous arthritides
No of pats.	12	14	12	15	17
C activity of synovial fluid					
Mean	93	103	108	93	100
Median	95	87	112	94	96
Range	49–130	60–131	79–140	72 127	60–133

Total 70 patients
Mean 99.2±2_.0 (S.D.)
Median 99.0
Range 49–140

Juvenile form only few joints involved (see 4 5).
Atypical polyarthritis (with involvement mainly of the large joints) 11 (in 3 associated with ulcerative colitis), postinfectious rheumatism 3 gouty arthritis 1 septic rthritis 1 and transient effusion f the knee-joint 1

group values lower than 56 (99.2–1 96 × _2.0 = 56 1) are considered depressed (at the 5% level)

Values from 56 to 140 were arbitrarily considered normal, although the values in the few normals studied fell within the lower range of the reference group (mean of 4 normals = 64 range 56–67) The data given by other workers (not using relative C units) appear to be compatible with a mean slightly lower than (10) or lar (1) to that of the present reference group tendency to somewhat low values in normal ovial fluid has been discussed elsewhere (4)

Table II *The latex titre of serum (latex) and synovial fluid (lat) at different synovial fluid complement (C) activ levels in 51 RA patients with a negative sensiti ed sheep cell test in serum (titre below 32)*

C activity of synovial fluid	No of pat	Frequency of elevated latex titres: Latex$_s$ and latex$_{sF}$	Latex$_s$ and/or latex$_{sF}$
> 62		1 23	1/23
56–62		1 2	1/2
43–55	8	8	4 8
< 43	18	7 18	12/18
> 56	25	2 25	25
< 56	26	10 26	16 26
			(χ^2 13 74 $p < 0.001$)

Fi out of the 16 patients had elevated titres (of t least 20) either only in serum or only in synovial fluid, the latex latex$_{sF}$ titres being < 10 40, 10 40 10 70, 10 70 and 70 < 10 (or 20/70 if fluid from the other knee was considered), respectively

Furthermore the mean (99._) and the variance (483) in the present reference group were similar to those (101.9 and 313 1 respectively) found in a group consisting of ten patients with osteoarthrosis, six with torn menisci, one with loose body of the knee-joint, and one with traumatic synovitis (4).

B *C activity of synovial fluid and RF positivity*

Of the 131 RA patients studied, the SSC test was positive in 80 and negative in 51 In rough one-third of the latter one or both of the latex tests proved to be positive It is noteworthy that latex positivity in this group of RA patients was confined almost exclusively to those patients in whom the C activity of synovial fluid was depressed (Table II) In most latex negative RA patients, the SSC$_s$ test had been consistently negative, but in most latex positive RA patients (1 out of 18) showing a negative SSC$_s$ test at the time of the present investigation this test had proved to be positive on at least one occasion

As already noted, RF positivity was generally demonstrated by at least two positive RF tests (see legend Fig. 1) The two-test positive group of patients agreed in synovial fluid C activity with the three test positive group whereas in the one-test positive group this activity tended to be lower In this latter group however gold-treated patients were over represented ($p < 0.05$), and in such patients the synovial fluid C activity as well as the RF titres tended to b. lower than in untreated" (4) In addition, although only one RF

titre was positive in the one-test positive group of patients, one of the other RF titres often approached the limit for positivity (Table II) and in four out of the six one-test latex positive RA patients, a positive SSC_a test had previously been demonstrated on at least one occasion.

Fig. 1 shows the distribution of the synovial fluid C activity values in all the arthritis patients. The series was divided into two groups, one RF positive and one RF negative. The close association between RF positivity and a depression of the synovial fluid C activity is evident.

Only in 12 out of 98 RF positive RA patients was the synovial fluid C activity found to be normal (Table III). There were no obvious clinical features nor other laboratory data distinguishing these 12 from those 86 in whom the C activity of synovial fluid was depressed, except that cases of fresh or early effusion predominated among the former. The marked difference in synovial fluid C activity between RF positive and RF negative patients was obvious also within the total group of RA, where only one-third of the RF negative patients (mostly having long-standing effusion) showed a lowering of the synovial fluid C activity in contrast to 88% of the RF positive (Table III).

The observed agreement in synovial fluid C activity values between adult and juvenile forms of arthritis was also evident in the group of RF negative RA. This group included the bulk of patients with juvenile RA (16 out of 25) and a few of the patients with adult RA (17 out of 106). Here the C values were very similar (p for the difference 0.70) in the two forms, and the frequency of depression of the C values was practically the same (5/16 and 5/17).

In RF negative RA, the synovial fluid C activity values were lower than in the reference group ($p < 0.001$) and higher than in RF positive RA ($p < 0.001$), the bulk of the C values corresponding to the lower range in the reference group (Fig. 1).

All but one of the patients in the group of SLE and SLE-like syndromes showed marked depression of the synovial fluid C activity (Fig. 1), and all except three out of the 14 patients were RF positive (Table III). The high frequency of RF positivity was ascribable to all patients having arthritis, most patients had SLE-like syndromes rather than typical SLE.

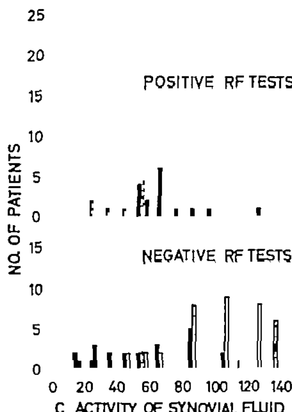


Fig. 1. The distribution of the complement (C) activity values of the synovial fluid in arthritic patients, 131 of whom had rheumatoid arthritis (filled columns), 70 various non-rheumatoid forms of arthritis (empty columns, see Table I), and 14 systemic lupus erythematosus (SLE) or SLE-like syndromes (hatched columns). The rheumatoid factor (RF) negative patients were negative in three separate RF tests, and of the RF positive patients 73% were positive in three separate RF tests and 93% in at least two tests (see Material and methods). The interrupted vertical is the line of division between non-depressed (or normal) and depressed C values (at the 5% level).

The synovial fluid C activity was subject to a sometimes considerable intra-individual variation, for example from joint to joint on the same occasion. Nevertheless, when synovial fluids were aspirated at the same time from two different joints of the same patient, the second set of fluids was found to agree in C activity with the first set. The observed frequencies of depressed C activity in the first and the second set of fluids were 0/14 and 0/14 in the reference group, 10/10 and 10/10 in the group of SLE and SLE-like syndromes, 2/6 and 3/6 in RF negative RA (intra-individual disagreement in only one patient), and

Table III *Agreement and disagreement between the synovial fluid complement (C) activity and the rheumatoid factor (RF) tests in 215 arthritic patients*

	No. of pats.	RF negative patients. C of synovial fluid		RF positive patients. C of synovial fluid	
		Normal[a]	Depressed[b]	Normal	Depressed
Non-rheumatoid arthritides	70	68	2 (0)	0	0
Rheumatoid arthritis	131	23	10 (6)	12	86 (75)
SLE and SLE-like syndromes	14	0	3 (3)	0	11 (10)
Total	215	91	15	12	97
Incidences		42.3	7.0	5.6	45.1

[a] The non-depressed range (56–140) considered normal.
[b] Depressed at the 5 % level, i.e. values below 56. Figures in brackets indicate patients with values depressed at the 1 % level, i.e. values lower than 43.

30/35 and 28/35 in RF positive RA. Essentially similar results were obtained when synovial fluids were aspirated from the same joint on different occasions. Thus the results shown in Fig. 1 and Table III were reproduced, group for group, when supplementary synovial fluids were studied.

C. C activity of synovial fluid in a group of patients with an obscure form of arthritis

In 13 patients, not included in the main series of arthritic patients because of uncertainty of the diagnosis, psoriasis was associated with RF negative polyarthritis, mostly of the rheumatoid type, affecting the distal interphalangeal joints. The synovial fluid C values in this group were non-significantly lower ($p \sim 0.10$) than those in true or definite psoriatic arthropathy (distal interphalangeal joints affected) and they resembled most closely those obtained in RF negative RA, depressed values being found in about one-third of the patients (4 out of 13).

DISCUSSION

The role of the C system in certain diseases, recently reviewed by Natvig and Winchester (9) is largely as a potential mediator of immune cell damage or inflammation. The present evidence of involvement or activation of the C system, a depression of the synovial fluid C activity in 7.% of the RA patients, may have a bearing on the pathogenesis in RA. However in cases of fresh or early effusion of the joint, the synovial fluid C activity has proved to be essentially normal in an appreciable proportion of patients with RF positive RA (4, 10). Accordingly if the involvement of the C system plays a pathogenic role in RA it may be that of a perpetuating mechanism. Also that possibility is difficult to defend, because of the sometimes considerable between-joint variation of the C values in long-standing rheumatoid effusion. These findings contrast with those in SLE and SLE-like syndromes, where the C values are consistently and markedly depressed even in instances of fresh or early effusion (4).

The mechanism(s) underlying the involvement of the C system in RA is still a matter of conjecture. An activation of the earlier steps of the C sequence through proteolytic enzymes cannot be ruled out, though it seems less probable (4, 6). More attractive seems to be the idea of an activation through antigen-antibody complexes or various forms of IgG-complexes (9). The demonstration of C3 and C4 (adjacent to IgG) in the rheumatoid synovial membrane (2, 11) suggests a binding of C to this tissue as the cause. Nevertheless, the increased anticomplementary activity of rheumatoid synovial fluids (4, 13) and the occurrence in these fluids of C-fixing cryoglobulins (8) and aggregated IgG (3), and of other types of complexes containing IgG focus interest on the synovial fluid itself. Marcus and Townes (8) suggested that the low C level of rheumatoid synovial fluid is ascribable to its content of (IgG-containing) cryoglobulins.

RFs are generally considered antibodies primarily directed to structurally changed (denatured) autochthonous IgG. Although not always demonstrably denatured, IgG seemed to be a common denominator in the above mentioned findings. I

iew of this relationship the close association between RF positivity and depression of the synovial fluid C activity suggests a common cause in the form of denatured IgG in the rheumatoid synovial membrane and/or fluid. This close association also strengthens the previously pointed out need for a clarification of the nature of rheumatoid IgG (11).

In the group of RF negative RA only one third of the patients showed depression of the synovial fluid C activity compared with about 90% of the patients with RF positive RA (Table III). Similar observations suggested to Vaughan et al. (14) that RF negative and RF positive RA could possibly be different entities. It has been shown by Dixon (1) that in adult RA, with mostly long-standing sero-negativity development of sero-positivity which appeared in one-third of the patients, did not occur until arthritis had existed for several years (mean 10.3 range 2–23 years). Similar observations have been made in juvenile RA (5). Whether or not at follow-up studies the RF tests may turn positive predominantly in so far RF negative RA patients, in whom the synovial fluid C activity had been shown to be depressed, remains to be determined.

The low frequency of depressed synovial fluid C values in RF negative RA, and the close association between depressed C values and positive RF tests together with the much larger amplitude of the latter tests, make the determination of the synovial fluid C activity less suitable as a routine diagnostic tool.

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CHEMOPROPHYLACTIC EFFECT OF PHENYLETHYLBIGUANIDE (DBI) ON INFLUENZA A2 AND INFLUENZA B VIRUS INFECTIONS IN DIABETIC PATIENTS

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Abstract The chemoprophylactic effect of phenylethylbiguanide (DBI) has been studied in 183 adult diabetics attending diabetic out-patient department during the outbreak of an influenza A2 and influenza B virus epidemic in Helsinki in 1965 Paired serum specimens were obtained from 53 patients in the DBI group and 130 in the non-DBI group with an interval of four weeks to two months for the titration of CF and HI antibody of influenza A2 and B. Symptoms and signs of acute respiratory disease (ARD) which occurred between the samplings or prior to the first sampling were recorded. The rate of total ARD was slightly and the rate of ARD with symptoms of typical influenza significantly lower in the DBI group than in the non-DBI group. The rate of ARD caused by influenza A2 was 8% in the DBI group and 18% in the non-DBI group, the difference between the two groups being statistically significant, whereas no significant differences were observed in the rate of ARD caused by influenza B virus. Influenza A2 virus caused 4/19 (21%) of ARD in the DBI group and 23/60 (38%) of ARD in the non-DBI group.

Among the antiviral agents which have been introduced during recent years, the biguanide derivative N^1,N^1-anhydrobis(β-hydroxyethyl)biguanide hydrochloride (ABOB) has been investigated in the treatment of various human virus infections (1 2, 3 8, 19) and has been claimed to be clinically effective in the chemoprophylaxis of influenza virus infections (4 16). Additional biguanides have also been tested in tissue cultures (7), although no evidence has been presented regarding the activity of these compounds in vivo. Quite independently during the past ten years, chemically related biguanide derivatives have been extensively used as orally effective hypoglycemic agents in the treatment of diabetes. This chemical relationship suggested that a chemoprophylactic effect might be exerted fluenza epidemic among diabet p with phenylethylbiguanide (DBI) port describes the frequencies of disease and serologically confirm infections among two groups o treated with DBI and the other rec n hypoglycemic drugs during an influenz in Helsinki.

MATERIAL AND METHODS

The present study was initiated in late February 1965 shortly after the onset of an influenza A2 (12, 13) and influenza B (9) epidemic. The original material consisted of 189 adult diabetics (mean age 58 years, range 17 to 78 years) who were being treated at the Out-patient Department of the Maria Hospital in Helsinki However nine diabetics who had been vaccinated against influenza during the epidemic were excluded from the series, and three patients with both an influenza A2 and B virus infection were each counted twice. Therefore the final number of patients was 183 They were divided into two groups according to the treatment received. As shown in Table I, the first group consisted of 53 diabetics treated with DBI alone or in combination with other oral agents or insulin (DBI group), and the second group consisted of 130 diabetics treated with sulfonylurea drugs, insulin or dietary regimen only (non-DBI group). The daily dose of DBI varied from 25 to 100 mg, average 58 mg. In 66% of the patients DBI was administered in the form of timed disintegration capsules. The mean age of the DBI group was 57 years, and that of the non-DBI group 58 years.

The patients received sugar-free diet with carbohydrate content not exceeding 200 g per day but quantitative calculation of the diet was not carried out. The control of the diabetes was in most cases satisfactory and there appeared to be no significant differences between the groups in this respect.

Table I. *Distribution of diabetic patients receiving phenethylbiguanide (DBI group) and of diabetic controls (non-DBI group) according to treatment*

Treatment	DBI group	Non-DBI group
Insulin	25	44
Tolbutamide	20	50
Chlorpropamide	3	4
Carbutamide	—	4
Carbutamide – tolbutamide	—	1
Insulin – tolbutamide	1	—
None (diet)	4	27
Total	53	130

All serum specimens for serologic studies were collected during a 4-month period from late February to early June. Paired sera were obtained from each patient with an interval of four weeks to two months. At the time of collection of the first serum the patients received a questionnaire to be filled in and returned at the time of the second sampling. This questionnaire was intended to provide information on the occurrence of signs of actual respiratory infection or on possible recent vaccination against influenza. If [illegible] patient showed two or more of the symptoms rhinitis, sore throat, cough or fever he was considered to be suffering from acute respiratory disease (ARD). Only those acute respiratory infections which occurred in the interval between samplings or during the three-week period prior to the first sampling were recorded. Acute respiratory symptoms associated with fever and definite aching of muscles were taken to [illegible] typical influenza.

The titration of complement-fixing (CF) antibody against influenza A2 and B [illegible] as carried out according to Sever microtechnique ([illegible]). The antigens of influenza A[illegible] and B used in the hemagglutination inhibition (HI) tests were prepared from strain A[illegible] Finland 1 6[illegible] isolated during the actual epidemic, and strain B Johannesburg 33 [illegible]. All sera were treated with periodate for the HI test. A four *fold or greater rise in the CF and or HI antibody titers* was taken to indicate infection. A CF titer $\geq$1 128 in the first serum specimen also indicated a recent infection. The serologic tests were recorded without knowledge of the clinical data or the treatment schedule of the patients.

The degree of statistical significance of the differences of the values obtained was determined by Student' [illegible] test.

Table II. *Rate of acute [illegible]espiratory disease (ARD) with and without typical symptoms of influenza in the two groups studied during the 4-month period*

Clinical category	DBI group	Non-DBI group
Typical influenza[a]	2 53 (4 [illegible])	19 130 (15 [illegible])
ARD without typical symptoms of influenza	16 53 (30 [illegible])	41 130 (32 [illegible])
Pneumonia	1 53 (2 [illegible])	0
Total	19 53 (36 [illegible])	60 130 (46 %)

[a] Definitions in Material and methods.

Table III. *Frequency of serologically verified influenza A2 and influenza B virus infections in the two groups according to clinical category*

Clinical category	Etiology	DBI group	Non-DBI group
ARD	Influenza A2	4/53 (8 %)	23/130 (18 %)
	Influenza B	6/53 (11 %)	12 130 (9 %)
No ARD	Influenza A2	4/53 (8 %)	7/130 (5 %)
	Influenza B	2/53 (4 %)	1/130 (1 %)

RESULTS

The incidence of acute respiratory disease among the 183 diabetics observed was high (43 %) during the 4-month observation period. As shown in Table II the rate of total ARD was lower in the DBI group (36 %) than in the non-DBI group (46 %) but the difference was not statistically significant, whereas the rate of clinically typical influenza was significantly lower ($p < 0.01$) in the DBI group (2/53) than in the non-DBI group (19/130)

According to serologic results, the rate of influenza A2 infection among all diabetics was 38/183 (21 %). In the DBI group it was lower (15 %) but not significantly less than in the non-DBI group (23 %). The rate of influenza B infection was [illegible]1/183 (11 %), and there was no significant difference between the DBI and non-DBI groups. When the infection rates were calculated separately among patients with and without ARD, the rate of influenza A2 infection was significantly lower ($p < 0.05$) in the DBI group with ARD (8 %) than in the non-DBI group with ARD (18 %), as indicated in Table III. In the patients without ARD the rate of influenza A[illegible] was slightly lower in the non-DBI group. In the rates of influenza B virus infection no significant differences were observed between the DBI and the non-DBI groups, irrespective of the presence of ARD

Four out of 19 cases with ARD (21 %) in the DBI group were influenza A2 infections, while 23/60 (38 %) of the ARD cases in the non-DBI group were influenza A2 infections. Influenza B

virus caused 6/19 (32%) of the cases of acute respiratory disease in the DBI group, and 12/60 (20%) in the non-DBI group

DISCUSSION

N[1]N[1]-anhydrobis(β-hydroxyethyl)biguanide hydrochloride (ABOB) is the only biguanide derivative which so far has been extensively investigated both in experimental virus infections and in the prophylaxis and treatment of human virus infections (10, 11). The results have been conflicting, and on the basis of carefully controlled studies Stuart-Harris and Dickinson (18) in their review of the chemotherapeutic activity of various agents in human virus infections, concluded that ABOB "is inactive in the treatment of the common virus infections in man or that, if not wholly inactive, the benefit conferred in self-limited infections is not worthwhile in clinical practice More recently however Swedish study group (4) investigated the prophylactic effect of ABOB in different dosages in over 5 000 industrial employees during an influenza A2 epidemic in 1965 and found a statistically significant effect on the clinical manifestations of influenza with a dosage of 800 mg twice a day while the effect with the previously used dosage of 400 mg twice day was less pronounced. While the chemical and pharmacodynamic properties of ABOB are comparatively well known (9) the mechanism of the possible antiviral action of this drug remains obscure.

The present study suggests that other chemically related biguanide derivatives may also have some effect in the chemoprophylaxis of influenza virus infections in man. Treatment with phenylethylbiguanide (DBI) reduced the total rate of ARD in diabetics during an influenza A2 and influenza B epidemic, the effect being more pronounced in patients who had ARD with clinical symptoms of typical influenza. Furthermore DBI significantly reduced the rate of serologically confirmed influenza A2 infections among ARD patients. Certain factors, however must be taken into consideration in evaluating the effect of drugs or vaccines in epidemic infections. Variability in the different epidemics is thus known to affect chemoprophylactic studies of this type, and a low morbidity will make evaluation of the treatment difficult. The conditions of the present study would seem favorable however insofar as the study was performed during the peak of an epidemic with a high incidence of morbidity as observed in the present study and confirmed by earlier reports (5 12, 13). Another factor which may occasionally affect chemoprophylactic studies is neglect on the part of the patients to take the prophylactic treatment prescribed. In the present study all patients were diabetics who were used to taking their medication regularly and in whom the control of diabetes remained on a satisfactory level during the period of study There would therefore seem to be little likelihood of this type of methodological error Finally the control of diabetes was, as far as could be estimated, similar in the group of diabetic patients treated with DBI and in the diabetic subjects treated by other means, and the two groups were comparable in this respect also

The data presented indicate that additional studies regarding the antiviral properties of hypoglycemic biguanide derivatives are justified in diabetic patients afflicted with various types of virus infections, particularly influenza A2. An evaluation of these compounds in non-diabetic subjects would probably also be feasible in addition to cell culture and animal experiments, since DBI and related biguanides, which do not affect the secretion or release of insulin, and probably depend for their hypoglycemic action mainly on the promotion of intracellular glucose metabolism in peripheral tissues, have no hypoglycemic effects in the non-diabetic individual (14 17) Moreover extensive clinical experience with hypoglycemic biguanide derivatives indicates that these compounds are essentially non-toxic, although gastrointestinal side-effects will probably occur more often with this type of medication than with ABOB and may occasionally necessitate discontinuation of the treatment.

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HEMODYNAMIC RESPONSES TO OXYGEN BREATHING AND THE EFFECT OF PHARMACOLOGICAL BLOCKADE

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Abstract. Breathing of 100% oxygen at 1 atmosphere for 20 min in healthy subjects produces concomitant cardiac depression and vasoconstriction. These adverse hemodynamic effects abolish the expected rise in general and regional oxygen transport. Pharmacological blockade of various types proved phenoxy-benzamine and atropine to be the only agents effective in preventing cardiac depression. An increase of total oxygen transport thereby occurred. In contrast vasoconstriction and blood pressure rise followed oxygen breathing irrespective of the type of pharmacological blockade applied. Consequently regional oxygen transport remained unaltered. The study provides evidence that cardiac depression and vasoconstriction as precipitated by oxygen breathing are separate and independent effects and that both are mediated via metabolic pathways.

Oxygen administration is known to produce marked hemodynamic effects as reflected by cardiac depression and vasoconstriction (8 17 20, 21). These effects obviously counteract the supposed benefits expected from oxygen treament of heart disorders and have made it necessary to reevaluate this treatment (12, 13 18).

The present work was undertaken to assess the hemodynamic effects provoked by oxygen breathing and their modification by various types of pharmacological blockade. As several basic mechanisms of this hemodynamic response are still poorly understood, these mechanisms were given special consideration. The study thus provides information as to the relationship between the cardiac and peripheral circulatory effects and to how these are mediated. Also possible measures are suggested for preventing the cardiac depression induced by oxygen breathing.

MATERIAL AND METHODS

Because the study aimed at revealing basic mechanisms, only healthy subjects were included. A total of 23 individuals were investigated, their ages ranging from 15 to 59 years.

The experiments started with one hour rest in the supine position. Thereafter determinations during air breathing were made of cardiac output, blood pressure, heart rate, calf blood flow and blood gases. Then the subject breathed 100% oxygen at 1 atm from reservoir delivering 6–10 l/min. The above determinations were repeated within the 10th–15th min of the oxygen breathing.

Cardiac output was determined by the dye dilution technique. Indocyanine-green was injected in the right atrium and brachial arterial blood was thereafter sampled continuously through cuvette densitometer with constant-rate, motor-driven syringe. Blood pressure was usually recorded intra-arterially but sometimes by the ordinary cuff method. In the latter instance, mean blood pressure was calculated as diastolic pressure plus 40 of the pulse pressure. Calf blood flow was obtained by means of venous occlusion plethysmography employing water-filled plethysmographs with the water temperature kept at 32 C (10). Arterial pO_2 was determined with Clark electrode (6), pH and pCO_2 by the Astrup technique (2) Oxygen saturation was measured by means of photometer (9).

The various types of pharmacological blockade were administered as follows. Hydralazine (Apresoline® Ciba) and phenoxybenzamine (Dibenzyline® Smith, Kline & French Labs) were given perorally over some days prior to the investigations This made it possible to check that the clinical effect was adequate. Hydralazine was employed in order to stimulate the heart and to oppose direct smooth muscle constriction of the peripheral arteries. It has been suggested that hydralazine acts via beta-adrenergic receptors (1) Phenoxybenzamine was used to induce selective blockade of the alpha-adrenergic receptors. A similar selective blockade of the beta-adrenergic receptors was obtained by intravenous injection of either alprenolol (Aptin® Hässle) or propranolol (Inderal® ICI) The first agent is known to exert a weak stimulation of these receptors In order to study the intrinsic heart function (11), alprenolol and atropine were administered in combination by the intravenous route

Table I. *Mean arterial blood gas values during oxygen breathing*

Environmental pressure (atm.)	Gas inhaled	pO_2 (mmHg)	SO_2 (%)	pH	pCO_2 (mmHg)	O_2-content (vol.%)
1	Air	87±1	96±0	7.42	35±0	19.5
1	Oxygen	548±3	99±0	7.43	34±0	20.9

Table II. *Mean hemodynamic values during oxygen breathing in normal subjects. Per cent change given in brackets*

Environmental pressure (atm.)	Gas inhaled	Cardiac output (l/min)	Heart rate (beats/min)	Stroke volume (ml/beat)	Blood pressure (mmHg)	Mean BP (mmHg)	Peripheral resistance (U)	Calf blood-flow (ml/min/100 ml tissue)
1	Air	5.7±0.5	67±1	84±2	126/79	95±1	18±0	2.22±0.18
1	Oxygen	5.0±0.4	63±1	78±4	131/83	102±	22±0	1.80±0.11
		(−12)	(−5%)	(−5%)		(+7%)	(+20%)	(−19)
		−5	13	−5	−13	−13	−13	13
		p 0.005	$p<0.005$	$p<0.05$		$p<0.005$	$p<0.005$	$p<0.005$

RESULTS

Oxygen breathing produced the predicted increase of arterial oxygen tension and saturation without concomitant change of the arterial pCO_2 and pH (Table I). This suggested that the hemodynamic effects were solely caused by the 1.4 vol.% in in the arterial oxygen content.

Breathing of 100% oxygen at 1 atm in the subjects produced a definite hemodynamic response (Table II), which was of the same direction although of different magnitude among the individuals. There occurred a 12% decrease of the cardiac output as reflected by a 5% reduction of both heart rate and stroke volume. The peripheral blood flow fell by 19% vasoconstriction thus superseding cardiac depression. Consequently the mean blood pressure increased by 7%.

Table III. *Calculated arterial oxygen transport during oxygen breathing*

Environmental pressure (atm.)	Gas inhaled	Whole body arterial oxygen transport (ml/min)	Calf-muscle arterial oxygen transport (ml/min/100 ml) tissue)
1	Air	1 112±98	0.43±0.04
1	Oxygen	1 070±86	0.39±0.02

The hemodynamic events were not marked due to the weak stimulus employed. However the effects were consistent and considered of sufficient magnitude for further studies.

These hemodynamic changes obviously exerted an adverse effect upon the oxygen transport, which became less during oxygen breathing than during air breathing (Table III). The distinct reduction in whole body as well as in regional oxygen transfer is clearly illustrated by the individual responses (Fig. 1).

Administration of pharmacological blockade served a twofold purpose. One was to evaluate the mechanisms underlying the hemodynamic response to oxygen breathing, the other was to find possible measures for their prevention (Table IV).

Hydralazine, as expected, raised the initial level of cardiac output and heart rate but proved ineffective in modifying or abolishing the circulatory effects of oxygen. There is a feature of the hydralazine experiment to be noted, namely that the cardiac output fell in the presence of a constant heart rate.

Remarkable was also the response to oxygen breathing during alpha-adrenergic receptor blockade with phenoxybenzamine. The cardiac output remained unaltered as did the heart rate. This result was unexpected since alpha-adrenergic receptors are not present in the heart. With re-

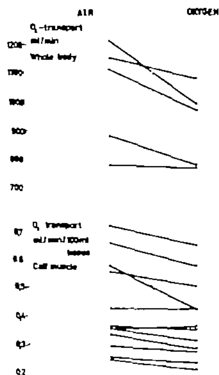


Fig. 1. Calculated calf muscle and whole body arterial oxygen transport during oxygen breathing. (Untreated subjects.)

gard to vasoconstriction and blood pressure rise, these responses were of identical magnitude and direction during blockade with both hydralazine and phenoxybenzamine.

Blockade of the beta-adrenergic receptors modified the circulatory effects of oxygen breathing in a manner which has to be considered in more detail.

The two agents, alprenolol and propranolol, were given intravenously in doses considered to provide the same extent of receptor-blockade. Alprenolol, however possesses the property of inducing a simultaneous stimulation of the beta-receptors. This is clearly reflected in the experiments. Cardiac depression was less, both before and during oxygen breathing, with alprenolol than with propanolol. The latter agent lowered the initial level of cardiac output markedly and the output did not decrease further in response to oxygen breathing. The interpretation of these results is difficult, but can be solved by means of the following arguments. If the two agents provided the same extent of receptor-blockade as originally intended and oxygen acted via these receptors, cardiac depression should be equal with both agents. When this is not the case, two explanations are possible. The stimulation of the receptors by alprenolol is in accordance with that achieved by hydralazine. For unknown reasons such a stimulation gives oxygen the opportunity to depress the cardiac output. However it cannot be excluded that propranolol gave a more potent beta-receptor blockade and that this prevented the oxygen

Table IV. *The effect of pharmacological blockade upon hemodynamics during oxygen breathing. Mean hemodynamic values after drug administration and during subsequent oxygen breathing*

Drug	Gas inhaled	Cardiac output (l/min)	Heart rate	Stroke volume (ml)	Mean BP (mmHg)	Peripheral resistance (U)	Calf blood flow (ml/min, 100 ml tissue)	Subj. no.
Hydralazine	Air	6.5	82	78	99	18	2.01	
	O_2	6.0	81	73	108	20	1.78	
Phenoxybenzamine	Air	5.6	76	73	89	16	2.03	
	O_2	5.6	76	73	96	17	1.78	
No drug	Air	5.5	78	71	70	13	3.06	
Alprenolol	Air	5.3	66	80	69	13	2.73	2
	O_2	5.1	65	79	81	16	2.20	
No drug	Air	5.7	54	106	80	15	1.29	
Propranolol	Air	4.4	48	92	78	16	1.12	2
	O_2	4.4	49	90	88	18	1.03	
No drug	Air	5.1	64	80	110	21	1.18	
Alprenolol atropine	Air	5.3	80	66	110	20	1.35	1
	O_2	5.3	75	71	113	21	1.18	

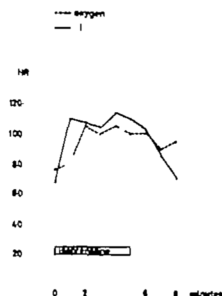


Fig 2 Influence of hyperoxia on cardiac response to isoprenaline.

Table V *The effect of pharmacological blockade upon oxygen transport during oxygen breathing Oxygen transport during air breathing without pharmacological blockade in brackets*

Drug	Gas inhaled	Whole body arterial oxygen transport (ml/min)	Calf-muscle arterial oxygen transport (ml, min/100 ml tissue)
Hydralazine	Air	1 270	0.39
	O_2	1 280	0.38
Phenoxy-benzamine	Air	1 085	0.39
	O_2	1 175	0.38
Alprenolol	Ai	1 035 (1 070)	0.53 (0.59)
	O_2	1 090	0.47
Propranolol	Air	860 (1 110)	0.22 (0.26)
	O_2	940	0.22
Alprenolol + atropine	Air	1 030 (995)	0.26 (0.23)
	O_2	1 130	0.23

response of the heart. The possibility that the circulatory effects of oxygen are mediated via these receptors therefore exists. A solution of this problem is reached by mere consideration of the oxygen-induced hemodynamic response. This con sists of cardiac depression and vasoconstriction fects which are recognized as those following inary treatment with beta-receptor blocking . Provided that oxygen exerted any action upon these receptors it had to function as a beta-receptor blocking agent itself This problem was studied simply by recording the heart rate in re sponse to a standardized amount of isoprenaline given intravenously As the increase of the heart rate was not abolished during oxygen breathing, the possibility of oxygen being a beta-adrenergic blocking substance could be ruled out (Fig. 2)

Regarding the peripheral circulatory effects caused by oxygen breathing, these were not prevented by beta-adrenergic blockade (Table IV) However the stimulating properties of alprenolol were again observed. During blockade with this agent vasoconstriction during oxygen breathing was more marked than during blockade with propranolol

Studies of the so-called intrinsic heart function yielded important results. The combined intravenous administration of atropine and alprenolol raised the initial levels of both cardiac output, rate and peripheral blood flow Subsequent oxygen breathing did not reduce the cardiac output. The heart rate fell but the stroke volume increased A small peripheral vasoconstriction and blood pres-

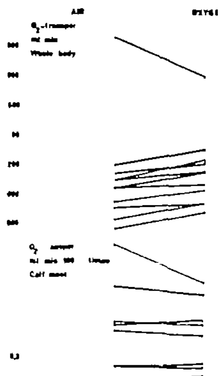


Fig 3 The effect of pharmacological blockade upon oxygen transport in calf muscle nd hole body during xygen breathing.

sure rise were registered. The agent obviously responsible for this result was atropine.

The oxygen transport during pharmacological blockade reflected the hemodynamic events observed during treatment with the various agents (Table V and Fig. 3). Total body transport of oxygen increased during treatment with phenoxybenzamine and atropine, clearly because cardiac depression was prevented. It was unchanged during treatment with hydralazine. Beta-receptor blockade apparently caused an increase of the transport. However in comparison with the transport during air breathing prior to the blockade, the transport was unchanged with alprenolol and lowered with propranolol treatment.

The peripheral, regional oxygen transport was not enhanced by any type of pharmacological blockade.

COMMENTS

The hemodynamic effects of oxygen breathing in healthy subjects, as presented in this report, are consistent with those observed in patients with hypoxia (13, 18), except for the observations by Storstein (17) that cardiac output increased in patients with cor pulmonale. Largely the results obtained in this study therefore, appear to be valid in both health and disease.

There are several problems regarding oxygen response to be considered, one of which is the underlying physiological mechanisms put to work by the oxygen stimulus.

It seems that three mechanisms are possible. Firstly cardiac depression may be the prime event, which initiates a compensatory vasoconstriction. This possibility can be ruled out, as vasoconstriction appeared also at a constant cardiac output. Secondly baroreceptor reflex may be involved, originating from vasoconstriction and blood pressure rise. Thereby inhibition of the sympathetic nerve discharge upon the heart would ensue and lower rate and output (1). The presence of such a reflex has been suggested (7 12), but is not supported by this study for the following reasons. Stimulation of the heart by means of hydralazine did not prevent or significantly diminish cardiac depression, which furthermore took place despite an unchanged heart rate. Also treatment with phenoxybenzamine, known to be without any direct cardiac action, abolished reduction of rate as well as output. The results of this report, therefore are in favour of the third possibility namely that the oxygen-induced effects upon heart and vessels are separate and independent events. This concept is consistent with observations made in open chest dogs that oxygen has a direct depressive cardiac action (19). Similarly oxygen has been found to exert a direct constrictor effect upon isolated arterial strips (15).

Regarding the mechanisms underlying the hemodynamic response another observation has to be considered. As mentioned, cardiac output fell during therapy with hydralazine, but not with phenoxybenzamine. In all other respects the circulatory responses were identical. The explanation of this remarkable event can probably be found by considering the peripheral effects of these agents. Both, in different ways, oppose arteriolar constriction. Hydralazine has, however only a weak action upon the veins (1) In contrast, phenoxybenzamine markedly affects the capacitance vessels in such a way that circulating blood volume becomes augmented (22). In the supine subject this may cause an increased venous return and thereby prevent cardiac depression. Possibly this may explain the results obtained when subjects treated with phenoxybenzamine are breathing oxygen. Such a view is supported by studies showing venous tone to be markedly influenced by variations of blood gases (5). Moreover both retinal arteries and veins have been demonstrated to undergo constriction in response to oxygen breathing (16).

This study also revealed that cardiac vagolysis with atropine was able to prevent cardiac depression following oxygen, an observation made previously by Daly and Bondurant (7) The present study furthermore demonstrated that the oxygen stimulus did not act via the beta-adrenergic receptors of the heart, and that oxygen itself did not produce any kind of blockade of these receptors.

Two conclusions can therefore probably be drawn. Firstly evidence is presented that the oxygen effects upon the heart are mediated via metabolic pathways and not via presently known receptor systems. Secondly cardiac depression in response to oxygen breathing can be prevented by treatment with either phenoxybenzamine or atropine.

Due to the adverse hemodynamic effects provoked by oxygen breathing, the total oxygen

transport did not increase in this series. This was also observed by Storstein (17), who noted a small, but not significant increase in normals and in patients with heart disease. In his study the normal subjects were exposed to oxygen breathing for 30 min, five out of 12 cases showing an appreciable reduction in oxygen transport, while the remainder showed a rise. Similarly only five out of 11 patients with heart disease had an increase in arterial oxygen transport although the exposure to oxygen breathing lasted for 60 min.

The present study also proved atropine or phenoxybenzamine to be the only agents effective in providing an augmentation in arterial oxygen transport. These findings may be of significant clinical importance in arriving at an effective form of oxygen treatment.

As shown, vasoconstriction attenuated the expected rise in peripheral oxygen transport, a finding in accordance with those of Bird and Telfer (4) who employed hyperbaric oxygenation. In the present study vasoconstriction occurred irrespective of the type of pharmacological blockade applied. Consequently the regional oxygen transport remained unaltered. It is unknown to what extent these observations are valid for other vascular beds, although wide variations in oxygenation of the coronary arterial blood have failed to significant changes of the myocardial metabolism (14).

It follows from the *experiments with pharmacological blockade that vasoconstriction as provoked by oxygen breathing is probably mediated via a metabolic pathway.*

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CORONARY ANGIOGRAPHY AND ANGINA PECTORIS

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Abstract. Selective coronary angiography has been performed in 35 consecutive patients admitted for anginal pain. Five patients had patent coronary arteries, whereas nine proved to have congenital anomalies of the coronary arteries. Thus about 40% of the patients did not have the expected coronary atherosclerosis. Among the twenty-one patients having obliterative coronary artery disease only six suffered from lipid metabolic disturbances. In these patients both the diffuse and the occlusive type of coronary artery disease was observed, indicating the influence of additional factors in precipitating the arterial injury. The majority of the patients with acquired coronary artery disease had normal blood lipids. A distinct discrepancy was observed between current criteria of true angina pectoris and demonstrated changes of the coronary arteries. Correlation between electrocardiographic changes and coronary artery obstruction was satisfactory.

The advent of coronary angiography (1–10) has been of prime importance in permitting an accurate estimate of coronary artery obstructions. Largely the changes expected to accompany ischemic heart disease have been demonstrated (3, 4, 8, 1., 15). Of much greater significance, however has been the finding of completely patent coronary arteries in a considerable number of cases (3, 4, 6, 14), even in patients who died from myocardial infarction (7).

These important observations are supported by the present report, which presents the results obtained by selective coronary angiography in a series of patients consecutively admitted for anginal pain. The series is remarkable for its considerable content of congenital coronary artery anomalies and for the discrepancy noted between current clinical criteria of angina pectoris and the observed changes in the coronary arteries.

MATERIAL AND METHODS

Patients with ordinary angina pectoris are seldom admitted to University Clinics, except for special reasons. In the present series the majority was admitted due to the combination of anginal pain and young age, especially when this coincided with female sex. Another reason was atypical pain or pain out of proportion to clinical findings. Finally some patients with incapacitating pain were referred for surgical revascularization of the myocardium or direct reconstructive arterial surgery of the coronary vessels. This series may thus be regarded as selected, although it contains only the kind of patients with chest pain encountered in every other series of angina pectoris.

The patients went through a routine clinical examination including special study of blood lipids and glucose tolerance. When the 12-lead electrocardiogram at rest was normal, the patients were exposed to a hypoxia test or an exercise tolerance test. The latter was performed by means of Master's two-step test or bicycle ergometry.

Selective coronary angiography was carried out according to the technique proposed by Judkins (10). In each case the three major coronary arteries with their tertiary branches were well visualized.

Two complications were encountered. One man had severe angina during the examination, but recovered rapidly afterwards without signs of myocardial injury. A young female acquired an iliac artery thrombosis on the side of catheterization. The resultant limb ischemia was transient, and the subsequent claudication has been negligible. No reconstructive surgery has been contemplated because the patient's main disease is considered to be an arteritis.

A study was thus made of 35 patients consecutively admitted from January 1968 to June 1969 (Table I). Nearly half the patients were females, and fifteen were below 40 years of age.

RESULTS

For further analysis the patient series was divided into a group with normal and a group with abnormal electrocardiograms at rest.

The first group comprises 17 patients, seven of whom had a positive exercise tolerance test and eight of whom obtained relief from their chest pain by nitroglycerine (Table II). The diagnostic

Table I. *The series of patients referred for selective coronary angiography due to chest pain*

Sex	No.	Mean age	Age group 20–29	30–39	40–49	50–65
♂	21	46	3	5	5	8
♀	14	38	4	3	3	4
Total	35	43	7	8	8	12

Table II. *The additional diagnostic yield from coronary angiography in 17 patients with chest pain and normal electrocardiogram*

Sex	No.	Exercise test	Positive nitroglyc. effect	Pathologic angiogram
♂	10	5	6	9
♀	7	2		5
Total	17	7	8	14

criteria of angina pectoris were thus met by less than half of the patients. By means of selective angiography distinct coronary artery disease could be demonstrated in fourteen patients.

Three of the seventeen patients had normal coronary angiograms. Two were women, aged 29 and 43 years, respectively. Both had for several years suffered from typical angina pectoris, but with inconsistent effect of nitroglycerine and negative exercise tolerance tests. All the remaining clinical examinations were unrewarding in these two women, except for the presence of a thoracic scoliosis. The third patient with normal angiogram was a 62 year-old man who for many years had suffered from severe pains in the precordium and the left arm on effort. He had a marked symptomatic relief from nitroglycerine, but the exercise tolerance tests were repeatedly negative. The only pathology demonstrated in this patient was a left shoulder arthrosis.

Among the 14 patients with abnormal coronary angiograms, no less than four proved to suffer from congenital anomalies of the coronary arteries (Table III). This remarkable result has to be considered in detail.

The two patients with arteriovenous fistulas originating from the right coronary artery were similar in several respects. Both had recently been treated for subacute bacterial endocarditis. Both had for a long time suffered from typical angina pectoris, unrelieved by nitroglycerine and with negative exercise tolerance tests. In the man aged 29 years a faint continuous murmur was audible at the apex. Subsequent right heart catheterization revealed a small left to-right shunt at ventricular level. In the 22-year-old woman a continuous murmur was present at the second right interspace. Right heart catheterization disclosed a left-to-right shunt at the atrial level of 34% of the pulmonary circulation. In both cases the demonstrated coronary artery anomaly explained all the clinical findings.

The man aged 36 with hypoplasia of the right

Table III. *The detailed clinical and angiographic findings in 14 patients with chest pain and normal electrocardiogram*

Rca = right coronary artery. L. ram. circ.fl. = ramus circumflexus from left coronary artery. L. ram. desc. ant. = ramus descendens anterior from left coronary art.

Sex	Age	Angina	Nitro-glyc.	Exercise test	Coronary angiogram
Congenital anomaly					
♂	28	Typical	Neg.	Neg.	Fistula Rca, sinus coron.
♂	36	Atypical	Neg.	Pos.	Hypoplasia l Rca
♀	22	Typical	Neg.	Neg.	Fistula Rca, R. atrium
♀	55	Typical	Neg.	Pos.	Aneurysm from Rca + Lca
Diffuse narrowing					
♂	56	Typical	Pos.	Pos.	Diffuse narrowing Rca + Lca
♂	42	Typical	Pos.	Pos.	Diffuse narrowing Rca + Lca
Occlusive disease					
♂	29	Typical	Pos.	Pos.	Occl. of L. ram. circ. flex.
♂	52	Typical	Pos.	Pos.	Occl. of L. ram. desc. ant.
♂	44	Typical	Neg.	Neg.	Occl. of L. ram. circ.flex and ram. desc. ant.
♂	52	Typical	Pos.	Neg.	Occl. L. ram. desc. ant.
♂	53	Typical	Neg.	Neg.	Occl. branch of L. ram. desc. ant.
♀	48	Typical	Pos.	Neg.	Occl. L. ram. circ.fl. + ram. desc. ant.
♀	29	Typical	Neg.	Neg.	Occl. periph. branches of L. ram. desc. ant.
♀	36	Typical	Pos.	Pos.	Occl. Rca, stenoses L. ram. circ.fl. ram. desc. ant.

coronary artery had since early childhood suffered from anginal pain without convincing relationship to effort and without effect of nitroglycerine. The exercise test produced typical angina, but no changes in the electrocardiogram. An additional left heart catheterization showed the presence of a bicuspid, but competent aortic valve. The 55-year-old woman had similarly complained of typical angina pectoris since youth. A faint continuous murmur was audible at the second right inter space. She did not obtain symptomatic relief from nitroglycerine, and the exercise test produced pain, but no electrocardiographic changes. Coronary angiography revealed an aneurysm composed of the conal branches from both coronary arteries. The aneurysm was situated at the root of the pulmonary artery and emptied into the latter. The aneurysm was successfully removed at the oper ation.

There were ten patients with acquired coronary artery disease. In two men with hyper-beta-lipoproteinemia and severe angina the angiography showed diffuse obliterative lesions without conspicuous stenoses or occlusions. In the remaining eight patients typical angina pectoris was present in all whereas only three had consistent symptomatic relief from nitroglycerine. Moreover only three had a positive exercise tolerance test. In two of them the test produced pain, but no objective electrocardiographic changes. This is remarkable considering the distinct occlusive lesions of the coronary arteries demonstrated in all the eight patients. Notable was also that all had normal blood lipids, except for a man aged 44 years with hyper-beta-lipoproteinemia.

In the 18 patients with abnormal electrocardiograms at rest, coronary angiography was of great assistance in arriving at the correct diagnosis (Table IV). There were twelve patients with electrocardiographic evidence of previous myocardial

Table IV *Findings in 18 patients with chest pain and abnormal electrocardiogram*

Sex	No.	Healed infarction	Positive nitroglyc. effect	Pathological angiogram
♂	11	7	5	10
♀	7	5	2	6
Total	18	12	7	16

Table V *The detailed clinical and angiographic findings in 16 patients with chest pain and abnormal ECG*

Sex	Age	Angina	Changes of ECG	Coronary angiogram
Congenital anomaly				
♂	35	Atypical	Q, T-inversions	Fistula L. ram. circ. fl., bronchial artery
♂	24	Typical	Post. infarction	Hypoplasia Rca
♀	37	Typical	ST deviations	Absence of major branch from L. ram. desc. ant.
♀	43	Typical	Ant. infarction	Hypoplasia Lca
♀	24	Atypical	Q T inversions	Single cor artery
Diffuse narrowing				
♂	53	Typical	L. ventr over load	Diffuse narrowing Rca Lca
Occlusive disease				
♂	37	Typical	Post. infarction	Occl. of Rca – stenoses Lca
♂	42	Typical	Post. infarction	Occl. of Rca L. ram desc ant.
♂	33	Typical	Post. lat. infarction	Occl L. ram. circ. fl. – sten. of Rca
♂	51	Typical	LBBB	Occl of Lca
♂	49	Typical	Q_1 and Q_{VL}	Occl. Rca
♂	43	Typical	RBBB, post infarction	Occl. of Rca – narrow Lca
♂	38	Atypical	Post. infarction	Occl. of Rca
♀	51	Typical	Ant. infarction	Occl of L. ram. circ.fl. sten L ram. desc ant
♀	24	Atypical	Anterolat infarction	Occl of L. ram. circ.fl. + narrow L. ram. desc. ant.
♀	56	Typical	Ant. infarction	Occl. of L. ram. desc ant

infarction. In six patients the electrocardiograms showed conspicuous Q-waves, T wave inversions and other changes, which were not specific of any distinct lesion.

Two of the 18 patients had normal coronary angiograms. One was a 51-year-old man admitted for surgical revascularization of the myocardium due to incapacitating and typical angina pectoris. He had a marked symptomatic relief from nitroglycerine. The electrocardiogram revealed pathological Q-waves in leads II and III, and slight signs of left ventricular systolic overload. An exercise test brought on pain, but no objective changes. The only pathology demonstrated in this patient was a thoracic spine scoliosis. The other patient was 51 year-old woman, who for sixteen years had suffered from angina pectoris and had gone through three proven myocardial infarctions. She





Fig. 1. Coronary angiogram from young woman with angina pectoris. There is so-called single coronary artery. It can be observed that the left coronary artery runs normal course from normally situated ostium. Just after its origin from aorta it gives off an apparently normal right coronary artery.

had marked relief from nitroglycerine, and the electrocardiogram showed signs of posterior and antero-septal myocardial infarctions. She did not smoke, and all other clinical findings were within normal limits. Left heart catheterization disclosed a bicuspid, but competent aortic valve.

Among the remaining 16 patients (Table V) a total of five, or nearly one third, proved to suffer from congenital anomalies of the coronary arteries. Again a detailed consideration is justified.

A 35-year-old man complained of low precordial pain, which mainly occurred some time after effort. The electrocardiogram showed variable, but usually marked systolic overload of the left ventricle. The negative T waves became nearly normal on exercise testing. He had no relief from nitroglycerine. Selective coronary angiography revealed a peculiar fistula from the circumflex artery to a bronchial artery and pathological collaterals around the septal branch of the anterior descending artery. Another man aged 4 years suffered a myocardial infarction one year prior to admission and had signs in the electrocardiogram of a previous posterior infarction. A very slight hyper-pre-beta-lipoproteinemia was demonstrated. Coronary angiography showed marked hypoplasia of the right coronary artery but nothing to explain the episode of infarction. A 37-year-old woman had noted unpleasant pain in the chest and left arm since childhood. Her blood lipids were normal, while the electrocardiogram showed slight ST depressions at rest and marked depressions on exercise. Coronary angiography demonstrated congenital absence of the non-septal branch of the anterior descending artery. Another woman aged 43 years had suffered from typical angina pectoris from the age of 20. There was a murmur indicative of mitral incompetence, and the radiogram revealed enlarged right ventricle and left atrium.

The electrocardiogram showed evidence of previous antero-lateral infarction. An exercise test produced pain as well as objective changes. The marked hypoplasia of the left coronary artery demonstrated by the angiography gave the explanation of her complaints. Of notable interest were also the findings in a 4-year-old woman who for

several years had suffered from dyspnoea and chest pain on effort. The symptomatic relief from nitroglycerine had been inconsistent. A faint systolic murmur was audible over the left second inter space. The heart showed a slight overall enlargement, while the electrocardiogram exhibited a pathological Q-wave in lead I and signs of systolic overload of both ventricles. The coronary angiogram showed the presence of a peculiar anomaly (Fig. 1). There was only one coronary artery namely the left one, originating from the aortic root. It departed from the usual site and immediately afterwards gave off a normal right coronary artery

Among the eleven patients with acquired coronary artery disease was a man with diffuse atherosclerosis of both coronary arteries. He had slight hypertension, but normal blood lipids.

In the remaining ten patients with occlusive coronary artery disease there were only three with lipid metabolic disturbances. Two men, aged 33 and 37 years, both had hyper-pre-beta-lipoproteinemia. The majority of these patients thus had a normal lipid metabolism.

Notable is the case of a 4-year-old woman who at the age of nineteen was treated for an anterolateral myocardial infarction and later on complained of dyspnoea and weak substernal pain on effort. Clinical data were indicative of arteritis. Coronary angiography revealed marked obstructive lesions of both main branches from the left coronary artery

Otherwise there is reason to notice the fair topographical correlation found between electrocardiography and coronary artery lesions.

COMMENTS

The essential and important observation made possible by coronary angiography is not the correspondence between ischemic heart disease and associated coronary artery obstruction (3 4 12). It is that a considerable number of patients with such disease have patent coronary arteries (5 7 14) and that obliterative lesions may be present in healthy subjects (15).

Firstly this has made it mandatory to distinguish between coronary heart disease and ischemic heart disease, which can only be demonstrated by disclosing myocardial ischemia (11). Secondly the fact that ischemic heart disease can occur with patent coronary arteries and in the absence of hypertension, valvular disease and myocarditis (8) has been a challenge to search for additional causes. Promising research is now being made on the occurrence of small vessel diseases (9) platelet thrombi and disturbed microcirculation (13) and derangement of the oxygen release from hemoglobin (7).

The present report contributes to a more varied view on the etiology of ischemic heart disease

The series comprised five patients with typical angina pectoris and patent coronary arteries. In three of them there was a marked symptomatic relief from nitroglycerine. The only pathology found was thoracic spine scoliosis and left shoulder arthrosis. Notable was also the presence of patent coronary arteries in a woman who had und gone three myocardial infarctions It is known that subendocardial infarctions are associated with normal coronary arteries (6), but this woman had electrocardiographic changes indicati e of transmural infarctions.

The remarkable finding in this series was the great number of patients suffering from chest pain due to congenital anomalies of the coronary arteries. They constituted nine of 35 cases, or 25 % of the series. These patients were on the average ten years younger than those suffering from acquired coronary artery disease. The result indicates the necessity of taking seriously anginal complaints in young patients and of carrying out selective coronary angiography This is the more important as several of the congenital anomalies are amenable to surgery Moreover the detection of very small left-to-right shunts is an indication for coronary angiography since this report shows that the underlying disorder may be an arteriovenous fistula of the coronary arteries.

This report provides an additional piece of important information. Of the 35 patients studied, a total of 14 or 40 % of the total series, had either patent coronary arteries or some kind of congenital abnormality of the latter Obviously such findings must affect conclusions based upon studies of coronary heart disease, in which coronary angiography has not been performed.

Also to be considered in this connection is the fact that only six of the 21 patients of the series with acquired coronary artery disease had lipid metabolic disturbances. Consequently the indications for undertaking selective coronary angio-

graphy must be settled without regard to the state of lipid metabolism.

There were four patients with hyper-beta-lipoproteinemia, two having diffuse and two occlusive lesions of the coronary arteries. Identical lipid metabolic disturbances thus produced two distinct vascular lesions, a finding indicative of possible additional etiologic factors. This is supported by the observations made in hypercholesterolemic subjects, in whom the extent of coronary artery obstruction did not bear a clear correspondence to the actual value of plasma cholesterol (2).

According to this report there is also reason to question the validity of current criteria of true angina pectoris. Typical pain is not to be trusted, nor the effect of nitroglycerine. A convincing symptomatic relief was observed in three patients with patent coronary arteries. Although the presence of myocardial ischemia on effort could not be excluded, it seems unlikely that any of them suffered from true ischemic heart disease. In contrast a consistent relief from nitroglycerine occurred in only ten out of 21 patients with marked coronary artery obstructions.

Similarly the exercise tolerance test gave a negative result in five of eight patients with normal electrocardiograms at rest, but whose coronary arteries were the seat of extensive structural disease.

It follows that severe failures may ensue from studies of coronary heart disease based exclusively upon clinical data.

Finally it may be stated that the observations made in this report indicate the superior significance of coronary angiography in the clinical study of coronary heart disease.

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THYROID ANTIBODIES AND THYROGLOBULIN LIKE PRODUCTS IN SERUM DURING ANTITHYROID THERAPY

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Abstract. In hyperthyroidism thyroid antibodies (thyroglobulin antibody and/or microsomal antibody) have been found in 30% of 44 patients examined before treatment was started, in 22% of 41 patients treated with antithyroid drugs, and in 21% of 34 patients who had previously undergone partial thyroidectomy. In a few patients there was a rather abrupt fall in the concentration of thyroglobulin antibody during treatment with antithyroid drugs. As this might possibly have been due to neutralization of the antibody in vivo, a number of sera which did not contain thyroglobulin antibody were investigated for circulating thyroglobulin. This investigation revealed thyroglobulin activity in the sera of 11 out of 18 patients who were receiving antithyroid drug therapy and in five out of 11 patients from whom antithyroid therapy had been withdrawn, but only in two out of 18 patients who had previously undergone partial thyroidectomy. Isolation experiments would suggest that the substance demonstrated might be genuine thyroglobulin, but—at least in some cases—with a lower iodine content than normal thyroglobulin. It is discussed whether the presence of this iodine-poor thyroglobulin-like protein and its breakdown products might not provide an explanation of the low PBI concentrations which are occasionally seen in patients during treatment with antithyroid drugs, once they have reached the euthyroid state.

The production of auto-immune thyroiditis in rabbits by Rose and Witebsky in 1956 (18) led to great interest in the subject of thyroid autosensitization, and it was soon discovered that in man three different thyroid-specific auto-antibodies may be found: antibody against thyroglobulin (14, 21); microsomal antibody against a microsomal antigen in the cytoplasm of the follicular epithelial cells (19); and the so-called CA-2 antibody against a colloid antigen which is different from thyroglobulin but not yet fully characterized (CA-2 = second colloid antigen) (2). To these may now be added long-acting thyroid stimulator (LATS), which also seems to have the character of an auto-antibody (1).

The immunological studies of the thyroid have been concentrated especially on Hashimoto's thyroiditis, as in this disorder autosensitization is ascribed a pathogenic role, although it is probably not the circulating auto-antibodies but more likely a coincident cellular immunity that is of importance. In hyperthyroidism LATS is apparently a factor of pathogenic significance, whereas the formation of the three other thyroid antibodies would seem to be more of a secondary phenomenon, the importance of which is as yet far from clarified. In particular little is known of these antibodies during treatment with antithyroid drugs, and the present investigation has therefore been specially designed to study this problem. The main purpose has been to try to compare the changes in the amount of thyroglobulin antibody and microsomal antibody which are to be found in the sera of patients with hyperthyroidism after treatment by partial thyroidectomy or antithyroid drugs. In order to obtain a better impression of the causes of the changes in concentration of thyroglobulin antibody that have been found, we have also investigated selected sera for thyroglobulin.

MATERIAL AND METHODS

The diagnosis of hyperthyroidism, i.e. a state characterized by hyperplasia of the thyroid gland, excessive secretion of its hormone, and raised basal metabolic rate, was made on the basis of the usual clinical and laboratory investigations, including the routine employment of tests using radio-iodine. The patients suffered from all degrees of hyperthyroidism, from very mild to very severe, although none was in thyrotoxic crisis. A total of 86 pa-

Table I. *Age and sex distribution of the 86 patients with hyperthyroidism*

Age	♂	♀	Total
11–20	1	2	3
21–30	4	12	16
31–40	0	8	8
41–50	1	14	15
51–60	4	14	18
61–70	8	13	21
71–80	1	4	5
Total	19	67	86

tients with hyperthyroidism, 67 women and 19 men, were included in the investigation. From Table I, which shows the age distribution of the patients, it appears that there were relatively few patients in their 30's, and the material thus falls into two main groups, a smaller group of young patients (18–30 years) and a larger group of older patients (41–70 years).

Forty of the patients were treated by operation, while 46 received drug treatment, 30 with methylthiouracil (MTU), five with carbimazole, six with first MTU and later carbimazole, and five with various other preparations (thiocyanide, methimazole and thyropropic acid). Drug treatment was considered preferable in mild cases, in those without enlargement of the thyroid gland, in patients who had been operated upon previously, in those with high titres of antibody and in patients with severe exophthalmus.

In 33 patients the concentration of thyroid antibodies was investigated both before treatment and once or several times during the drug treatment or after operation (generally 1–2 years after operation), but we have also included the results from 11 patients in whom the antibody concentration was measured only before the start of the treatment and a further 42 patients who were already receiving drugs or had been operated upon. All serum samples were investigated for thyroglobulin antibody and microsomal antibody. Only one sample from each of 47 treated patients (18 after thyroidectomy and 29 during or after drug therapy) was investigated for thyroglobulin activity as the method used was not developed until shortly before the follow-up investigation of the later patients. The determinations have been used merely to compare the conditions in the two groups which had received different forms of treatment.

Thyroglobulin antibody was demonstrated by passive haemagglutination ("tanned red cell technique"), using formalinized tanned sheep red corpuscles, coated with purified human thyroglobulin (6). After inactivation and absorption with sheep red cells the serum was titrated in five-fold dilutions, and 0.1 ml of each of these dilutions was then mixed in agglutination trays with 0.1 ml of suspension of the coated cells. After standing at room temperature for about two hours the cells settled and the agglutination result could be read off.

Microsomal antibody was demonstrated by a complement fixing technique using an extract of toxic thyroid tissue as antigen (15). In order to ensure the specificity of the reaction, all sera with which there was complement fixation were also investigated by means of immunofluorescent staining with acetone-fixed sections of thyroid tissue (3) and the antibody was accepted as microsomal antibody only when there was specific staining of the cytoplasm in the follicular epithelial cells.

The thyroglobulin activity of the serum was investigated by a so-called "reversed" haemagglutination technique (10), using formalinized tanned sheep erythrocytes coated with purified rabbit antibody against human thyroglobulin. Thyroglobulin antibody coated cells produced in this way will be agglutinated by thyroglobulin, or possibly other products containing the antigenic groups of thyroglobulin in bivalent or multivalent form which may be present in the serum. In this investigation the absorbed serum is titrated in three-fold dilutions, but apart from this the agglutination was carried out as described for thyroglobulin antibody. The method permits the demonstration of 0.03 µg thyroglobulin per ml, but as the lowest serum dilution used was 1:10 it was only possible to recognize concentrations in the serum corresponding to 0.3 µg thyroglobulin per ml.

Table II. *Occurrence of thyroglobulin antibody and microsomal antibody in untreated patients with hyperthyroidism, thyroidectomized patients (at least 1 year after operation) and patients who were receiving or had received antithyroid drugs*

	Untreated pats.		Thyroidectomized pats.		Pats. treated with drugs	
	No.		No.	%	No.	%
No antibodies	31	70	27	79	32	78
Thyroglobulin antibody only	6	14	2	6	1	2½
Microsomal antibody only	1	2	1	3	1	2½
Both thyroglobulin and microsomal antibody	6	14	4	12	7	17
Total	44		34		41	

RESULTS

The incidence of thyroglobulin antibody and microsomal antibody in the sera from untreated, thyroidectomized and drug-treated hyperthyroid patients is shown in Table II. In all three groups there was a remarkably small number of patients in whom there was evidence of autoimmunization. Whilst earlier investigations using the same methods have revealed one or more thyroid antibodies in the serum of 50–75% of untreated patients with hyperthyroidism (7, 9, 15), we have found

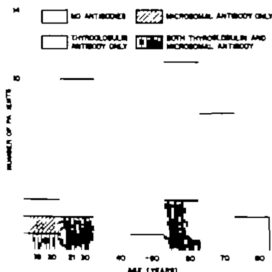


Fig. 1. Antibodies in 44 untreated patients with hyperthyroidism (37 women and 7 men) related to the age of the patient.

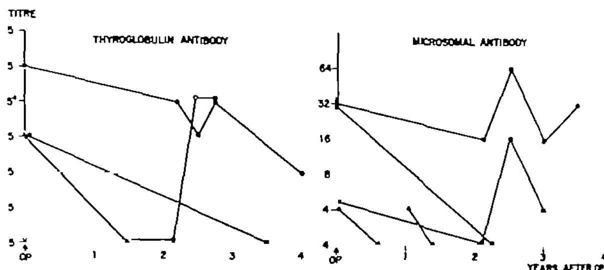
antibodies in only 30% of the 44 untreated patients investigated here. As was to be expected, the concentrations of antibody were low or moderately high. It is apparent from Fig. 1 that the findings in the small group of young patients were similar to those in previous studies (antibodies in 7 of the 13 patients under the age of 30 years) and the low incidence of antibodies is thus due to the fact that the present material has included a particularly large number of older patients with hyperthyroidism without evidence of autosensitization.

Thyroid antibodies were found in even fewer of the treated patients, in 21% of the patients following operation, and in 22% of those who were receiving or had received drug therapy but these findings do not differ significantly from those in the untreated patients. Otherwise it is difficult to make any comparisons between the three sets of results shown in Table II, as the presence of antibodies in some of the patients may have influenced the choice of treatment.

Despite the fact that no changes in the occurrence of antibodies resulting from treatment could be demonstrated for the groups as a whole, it is quite possible that there may have been changes in the presence of antibodies in the individual patients, and in those patients who were followed up with several investigations this was indeed found to be the case (Fig. 2). Thus the results from the few thyroidectomized patients who had thyroid antibody in the serum before operation showed a tendency to a fall in the antibody concentration or even a complete disappearance of the antibody within the first two or three years after operation. This is in complete accord with the results of previous, larger investigations, which have demonstrated that, in particular, the concentration of microsomal antibody often diminishes quite rapidly after partial thyroidectomy, while the concentration of thyroglobulin antibody either diminishes more slowly or possibly remains unchanged (7, 11, 13). However, the longer observation periods in some of the patients studied have revealed that one patient developed thyroglobulin antibody about two years after operation, while in two patients there would seem to have been a transient increase in the concentration of microsomal antibody about $2^1/_2$ years after the partial thyroidectomy.

The results from those patients who had been treated with drugs (Fig.) revealed a very variable pattern for the concentration of microsomal antibody, although as a rule there was a transient rise in the titre after the start of the treatment. These changes would seem to correspond to those which are observed after treatment with ^{131}I (5). The most remarkable finding after treatment with antithyroid drugs was, however, the marked fall in the concentrations of thyroglobulin antibody. In some patients this antibody seemed to disappear even more rapidly than is seen, for example, after thyroidectomy or in patients with myxoedema during treatment with thyroid hormone, where it is otherwise assumed that the immunological processes are to a very great extent inhibited.

When an antibody disappears remarkably rapidly, the question arises as to whether this might not be due to neutralization of the antibody in the circulation by its binding to the corresponding antigen. This is a natural question, as earlier investigations using an indirect method have suggested that at all events treatment with carbimazole may be associated with the occurrence of thyroglobulin or thyroglobulin-like substances in the serum (8). As it is technically difficult to determine whether there is complete or partial neutralization of a previously demonstrable thyroglobulin antibody in vivo, we have attempted to clarify the problem by investigating whether it was possible to demonstrate thyroglobulin activity in



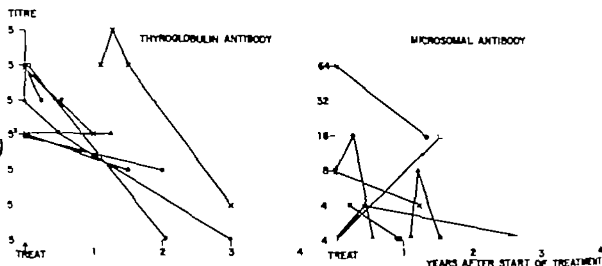


Fig. Changes in the concentration of thyroglobulin antibody and microsomal antibody following partial thyroidectomy and during treatment with antithyroid drugs.

a series of sera from patients in whom no thyroglobulin antibody had previously been present.

The results (Fig. 3) reveal that only two of the 18 sera from patients who had undergone thyroidectomy more than one year previously gave weak agglutination reactions with thyroglobulin antibody coated cells, while in contrast there were positive reactions—in several cases even marked reactions—in the sera of 11 out of the 18 patients receiving therapy with antithyroid drugs. If those patients who were treated with MTU are considered separately it is found that there was thyroglobulin activity in the sera of ten out of the 11 patients investigated. After withdrawal of the antithyroid drugs the conditions seem to some extent to return to normal, as six of the 11 sera in this group (and 5 of the 7 sera from patients who had received MTU) gave negative reactions. A rank-sum test reveals that the findings in patients receiving antithyroid therapy are significantly different from those in the patients who had previously undergone thyroidectomy ($p < 0.01$). Seen as a whole the findings in the patients receiving antithyroid therapy did not differ signifi-

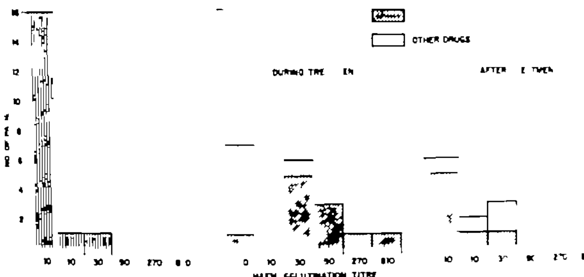


Fig. 3 Agglutination titres against thyroglobulin antibody coated cells in treated hyperthyroid patients in whom no thyroglobulin antibody could be demonstrated.

cantly from those in patients from whom such therapy had been withdrawn, but if only those patients who received or had received MTU are considered, then there was also a significant dif ference in this case ($p < 0.01$).

In order to obtain a more detailed characteristic of the agglutinating substances in the serum, frac tionation experiments were carried out with six sera which reacted particularly strongly In three of the sera the serum proteins were separated by filtration through a Sephadex G 200 column, and the agglutinating effect of the various fractions on thyroglobulin antibody coated cells was investigated. In all three cases the thyroglobulin activity was found to be localized to the first of the three main peaks which are normally seen, i.e. to the macroglobulin peak, where one also recovers purified human thyroglobulin which has been added to normal serum (Fig. 4). In three other sera the separation was carried out by ultracentrifugation in sucrose gradient, ranging from 10% to 37% sucrose (20). A Spinco centrifuge, model L 2, with swinging bucket rotor (SW 50) was employed, and centrifugation was carried out for 18 h at 35 000 r.p.m. In a similar manner the agglutinating effect was found in the lower fractions together with the high-molecular serum proteins, and in these cases again it was in the same fractions as human thyroglobulin is found in recovery experiments.

These results would thus seem to gi e grounds to believe that the protein which has been demonstrated is genuine thyroglobulin. As circulating thyroglobulin will be included in the ordinary determination of protein-bound iodine, its presence should be reflected in the PBI values, but—at least

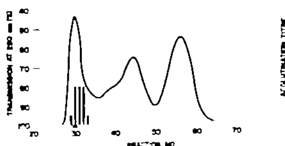


Fig. 4 Sephadex (G 200) filtration of 1 ml serum (T 2064) using phosphate-buffered saline (pH 7.2). The curve shows the transmission at 280 mμ, as registered by LKB uvicord with recorder. The columns indicate the agglutination titres of the individual fractions against thyroglobulin antibody coated cells (original titre of serum = 90).

in some sera—this was not the case. As the smallest amount of thyroglobulin which can be demonstrated by means of the technique used is of the order of 0.03 µg per ml, then the serum which, for example, had a titre of 810 should contain about 2 400 µg thyroglobulin per 100 ml. Even if one takes the iodine content of human thyroglobulin as only 0.31 % which is the lowest of the various values given in the literature (1), this concentration of thyroglobulin should increase the concentration of protein-bound iodine in the serum by about 7.5 µg per 100 ml However in the serum concerned, the total PBI concentration was only 1 0 µg per 100 ml and even when one takes into consideration the considerable inaccuracy associated with this calculation, it must be concluded that the serum did not contain normal thyroglobulin. The findings would suggest the presence of a protein which completely resembles thyroglobulin as regards antigenic configuration and molecular weight, but with a far lower iodine content.

DISCUSSION

The remarkably low incidence of thyroglobulin antibodies in the patients with hyperthyroidism investigated is difficult to explain. It is probably most natural to assume that this was due to a lack of sensitivity in the methods employed. As the formalinized erythrocytes were to be used for a number of different purposes, it was found necessary to attach great importance to ensuring that there was not the slightest tendency to spontaneous agglutination of the cells, and this has perhaps resulted in the demonstration of thyroglobulin antibody being slightly less sensitive than that in most other laboratories nonetheless, in routine determinations we have regularly found sera with titres of between 100 000 and 2 000 000 Failure of the method is thus unlikely to be the explanation of the low incidence of antibodies, and this is also confirmed by the fact that it would imply that two completely independent methods should both have failed furthermore it is only among the sera from the older patients that the incidence has been found to be especially low In addition the thyroglobulin determinations in the patients treated with MTU provide to some extent a check of the deter mination of thyroglobulin antibody as only one of the investigated sera which contained no thyroglobulin antibody did not contain thyroglobulin, and in this group accordingly there is the possibility of failure of the haemagglutination technique only in this one serum.

The investigation has otherwise demonstrated that during treatment with antithyroid drugs those patients in whom there was an antecedent thyroglobulin antibody in the serum often show a marked fall in the concentration of this antibody In patients in whom there was previously no thyroglobulin antibody it is found by contrast, that during this treatment—especially during treatment with MTU—it is often possible to demonstrate a thyroglobulin-like protein in the serum. Corresponding results have recently been reported by Roitt (17) who, by means of the sensitive radioimmunoassay also found raised concentrations of thyroglobulin in the sera of patients treated with MTU He explains the finding as due to increased production of TSH but does not seem to touch upon the question of the iodine content of the thyroglobulin.

It must therefore be concluded that a very rapid fall in the concentration of thyroglobulin antibody seen after the initiation of antithyroid therapy is not necessarily an expression of a weakening of the immunological processes, but that it might be the result of a direct neutralization of the circulat ing antibody It may perhaps be considered strange that a compound with the same antigenic groups as thyroglobulin should not give rise to production of antibody but it is unlikely that thyroglobulin is in itself an auto-antigen (16). Just as it is necessary in animal experiments to employ an adjuvant (Freund's adjuvant) in order to release autoimmunisation with thyroglobulin it would seem that an adjuvant is also necessary in man, and one of the great problems in the question of thyroid immunology is to discover which factors in man may have such an immunisation-provoking effect.

The presence of a thyroglobulin-like protein in the serum is not necessarily of merely theoretical immunological interest, but may also have a clinical aspect. Firstly it is not surprising that the iodine content of the protein which has been demonstrated seems—at all events in some cases—to be very low as one of the purposes of antithyroid therapy is precisely an inhibition of the iodine incorporation in the hormone precursors. The next question which arises is that of the biological ac-

tion of this thyroglobulin-like substance. As some diiodo or even iodine-free thyronine derivatives may well have a metabolic action, the presence of an iodine-poor thyroglobulin-like protein, and especially its breakdown products, might perhaps provide an explanation of the strikingly low PBI values which are occasionally observed in patients receiving antithyroid drug therapy when they have reached the euthyroid state. Christensen et al. (4) have found an increased T_3 ratio in patients treated with MTU and consider that this might explain the low PBI concentrations. However a number of these patients with reduced PBI had low serum concentrations of both triiodothyronine and thyroxine, and the assumption that the sera of these patients contain other less iodine-rich, active thyronin derivatives would therefore seem to fit in well with the picture, and supplement these findings. Even though in the present investigation we have found no obvious correlation between the concentrations of the thyroglobulin-like product and the protein-bound iodine, there is nonetheless food for thought in the fact that the patient whose serum reacted most vigorously with the thyroglobulin antibody coated cells (titre 810) was also the patient with the lowest PBI value (1.0 μg/100 ml).

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FAMILIAL MYOCARDIAL FIBROSIS

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Abstract. A family is described, suffering from myocardial fibrosis. The maternal grandmother died at an early age. The mother and one of her sisters likewise died young, probably from cardiac causes, and of the present generation seven of the ten members died acutely or after a period of heart failure between the ages of 22 and 53. Two postmortems have been performed. In one patient the postmortem showed extensive myocardial fibrosis without coronary sclerosis; in the other there was arteritis of several organs due to methylthiouracil therapy and likewise myocardial fibrosis (which undoubtedly contributed to the patient's death). No anatomical data are available on the remaining eight members of this generation; it is probable, however, that the five other deaths were due to the same disease. Of the three surviving patients, one has already developed cardiomegaly with auricular fibrillation, one is suffering from angina pectoris and auricular fibrillation, and the third shows no cardiac abnormalities but marked hypogonadism.

In 1949 Evans (4) described a family in which nine members died from intractable heart failure. They included a woman, some of her children, and some of her brothers and sisters. The usual causes—valvular diseases, hypertension, coronary sclerosis, amyloidosis, glycogen storage disease and Friedreich's ataxia, with their cardiac complications—were ruled out. Postmortem examination of a number of these cases disclosed diffuse fibrosis of the myocardium with secondary hypertrophy of the remaining muscle fibres. Evans described the syndrome as familial cardiomegaly. Similar cases have since been described by other authors (2, 3, 5, 6, 8).

We had the opportunity to study a patient with similar symptoms and findings, and it later appeared that our patient belonged to a family with an impressive number of cardiac deaths at a fairly early age.

MATERIAL

Case report

The patient (case no. 9) was observed in detail be[illegible] died at the age of 41. He was seen first in December[illegible] with complaints of dyspnoea and oedema. He was [illegible] but otherwise reported no definite complaints. The [illegible]toms had increased gradually.

At examination we found an accentuated [illegible] heart sound and a systolic murmur at the apex [illegible] crepitant rales. The blood pressure was 130/80 mmHg. The chest X-ray showed enlargement of the heart with signs of pulmonary congestion (Fig. 1). The ECG (Fig. [illegible]) disclosed auricular flutter with flat and negative T-waves in various leads. Ventricular ectopic beats were observed occasionally. The circulation time was increased: 22 sec with $MgSO_4$.

The laboratory data yielded little of value: the haemogram was normal, as were the blood electrolytes. Renal function tests, liver function tests, anti-streptolysin titres and serum enzyme levels were normal. The same applied to serum-cholesterol and lipoids; no L.E. cells and no cryoglobulins were found. Repeated blood cultures were negative. The sputum contained no bacteria. Antinuclear antibodies (ANF) were negative. The serum protein patterns were normal. Lung function tests: slightly decreased vital capacity of 3 660 ml (normal 4 475 ml), Tiffeneau 63% (normal 69%). Intravenous pyelograms normal.

A muscle biopsy disclosed some increase in the number of muscle cell nuclei, with row formation, and variability in calibre of the muscle fibres and degeneration of some fibres. Basophilic atrophic fibres were found scattered through the material, some contained fairly large nuclei (regeneration), while others showed degeneration. The arteriolar walls were markedly thickened. A skin biopsy was normal.

Conclusion of the pathologist: clearly pathological but no pathognomonic features. The picture might be consistent with myopathy or myositis but equally well with collagen disease or prednisone therapy.

Neurological examination: the electromyogram (EMG) showed increased excitability at rest. Mild paraesthesias were also found, but no other distinct changes. Conclusion: possibly incipient polyneuritis and—in view of the EMG—a myopathic picture or myositis of unknown origin.



Fig. 1. Thorax X-ray propositus, January 1963.

1 2 3

a

AVR AVL AVF V1 V2 V4 V6

1 2 3

b

AVR AVL AVF V1 V2 V4 V6

Fig. 2 a. Electrocardiogram of propositus. December 196.
Fig. b. Electrocardiogram of propositus. August 1964.



Fig 3 Survey of myocardial fibrosis. Connective tissue diffusely distributed throughout the myocardium. Azan, 35

The pressure curve of the right ventricle during heart catheterization showed the changes typical of rigidity of the heart wall (due to pericarditis, myocardial fibrosis or endomyocardial fibrosis and amyloidosis, i.e. an immediately postsystolic dip to below the base line, with subsequent end-diastolic plateau).

The pressure in the lung capillaries was increased (22 mmHg), indicating impaired drainage of blood from the left atrium to the left ventricle. Cardiac output was 4.1 l, the arterial oxygen saturation 94 %. Dye dilution curves (with indocyanine green) failed to demonstrate any shunts.

The condition was diagnosed as heart failure of unknown origin but probably resulting from degenerative myocardial disease. The apical systolic murmur was thought to be caused by relative mitral regurgitation. The patient was treated with digitalis and diuretics, which led to excellent improvement: the heart was reduced in size and the pulmonary congestion disappeared.

In May 1964 the patient was re-admitted with evidence of left and right heart failure. The apical systolic murmur was still present. The venous pressure was increased. The liver was palpable 4 cm below the costal margin. The circulation time was increased (20 sec).

Laboratory studies disclosed few abnormalities which the previous study had not demonstrated, increased SGOT (56 U) and LDH activity (450 U). The glucose tolerance test was slightly disturbed. Thyroid function was normal. Clinical treatment with digitalis and diuretics gave some improvement. At the end of this period in the hospital the SGOT, SGPT and LDH activities were substantially increased (260, 348 and 485 U respectively).

The patient's final hospitalization was in August 1964. He had oedema, was exhausted and dyspnoeic. The blood pressure dropped to 100/85 mmHg. Venous pressure was increased and the heart was markedly enlarged. The apical systolic murmur persisted. The liver was enlarged. A chest X-ray again showed marked cardiomegaly and pulmonary congestion. The ECG was obviously different from earlier recordings. There was still auricular fibrillation, but several leads (V 2 and V 4) now showed QS waves as often seen in myocardial infarction (Fig 6). This time the patient was suffering from intractable heart failure, he went into a state of shock, and died shortly afterwards.

Postmortem findings

The heart weighed 510 g (normal 300 g). It was dilated and flaccid and the tricuspid and mitral orifices were much too wide. Endocardium and valves were intact; the patency of the coronary vessels was adequate with only

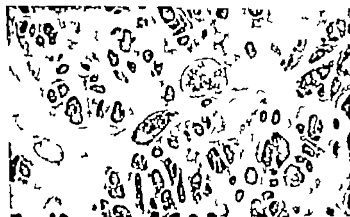


Fig 4 Heart. Every muscle fibre is enveloped in connective tissue. No vascular anomalies. H–E, 140.



Fig 5 Further details from Fig. 4. Central degeneration of muscle fibres. H-E, 350.

a few atheromatous plaques. Centrilobular congestion of the liver pulmonary oedema, ascites and hydrothorax completed the picture of heart failure.

Microscopic examination of the heart disclosed extensive fibrosis (Figs. 3, 4, 5 6 and 7). Many muscle fibres had been replaced by connective tissue. The fibrosis was occasionally seen in patches, but elsewhere individual muscle fibres had degenerated, with vacuolation, swelling and disappearance of myofibrils. The process seemed to start chiefly in the centre f the fibres. Reticulin and collagen deposits were found in and around these degenerative muscle fibres. Most veins were congested. The arteries showed no degenerative changes; no arteritis; no ...ications f myxoidosis The endocardium showed local ...elling and contained reticulin fibres and collagen, but there was no increase f elastin fibres. In view f the individual degeneration of muscle fibres, it was concluded that this process was one of primary degeneration of muscle fibres The cause of the condition remained obscure

Family study

It appeared that the propositus belonged to family in which many members had died at relatively early age, apparently from cardiac disease. Fig. 8 shows the pedigree of this family as far as it could be traced. 1 generation I the maternal grandmother died suddenly at the age of 26 the cause of death is unknown, but was probably due to cardiac arrest. The maternal grandfather died at the age of 90. A daughter of this couple—the mother of the large generation III—died suddenly at the age of 46; her death coincided to *the day* with that of one of her sisters, aged bout 48 Little is know about the paternal line. The paternal grandfather died when he was 59 years of age no further data could be traced. The paternal grandmother also died at the age of 50, of carcinoma. The son of this couple—the father of the large generation III—suffered from chronic bronchitis and died at the age of 54 from cor pulmonale. The members of generation I were *not* consanguineous; no information is available in this respect concerning earlier generations.

Generation III consisted of ten individuals, seven of whom died at ages ranging from 22 to 53 years.

Patient 1 was woman who died at 47 years of age she was described by Meyler et al. (7). This woman was admitted at the age of 46 for treatment of severe hyperthyroidism. She was given methylthiouracil, hereupon



Fig 6. Heart. Some myocardial fibres cut lengthwise show central degeneration. Azan. 350.



Fig. 7. Heart. Myocardial fibres surrounded by reticulin fibres. At one site reticulin fibres are seen to form a brush at the end of a muscle fibre. Silver impregnation of reticulin fibres, 350.

the symptoms disappeared quickly. Six months later she developed erythema of both legs and was treated with sulphonamide; symptoms of polyneuritis then became manifest and the patient was hospitalized. Although no immediate threat to life seemed to exist, she died suddenly. The postmortem disclosed typical features of arteritis, especially in the liver, kidneys (Fig. 9) and major nerves. The myocardium contained large areas of connective tissue (Fig. 10).

The diagnosis was methylthiouracil-induced arteritis in woman who had marked myocardial fibrosis since an early age. Meyler et al. (7) concluded: "Disturbance in cardiac function had been clinically demonstrated months earlier (fibrillation and dilatation) and it seems plausible that the final illness—the arteritis which also affected the myocardium to slight degree—overburdened the weakened heart and produced fatal disturbance in its function.

Patient 2 is male who died suddenly at the age of 22; no further data are available.

Patient 3 was woman treated elsewhere for heart failure. Cardiomegaly was observed in 1959. The patient deteriorated gradually over period of 3 years as result of intractable heart failure. Some 2 / years later the chest X-ray disclosed unmistakable cardiomegaly with pulmonary congestion. The patient died from heart failure aged 53. Unfortunately no postmortem examination as performed.

Patient 4 was nurse who like patient 2, died suddenly (aged 45). Her history mentions gastric complaints and gastric haemorrhage; the cause of death was obscure, and no postmortem was performed. The chest X-ray obtained some time before death disclosed no unequivocal abnormality of the heart.

Patient 5 was oman who died at the age of 40 from pneumonia (?). There are no further details available.

Patient 6, 53-year-old male, is still alive but under treatment elsewhere for heart failure. In 1959 he had an enlarged heart with auricular fibrillation and ventricular ectopic beats. He was being treated with quinidine sulphate, which resulted in temporary restoration of the sinus rhythm, but with an increased PQ interval of 0..4 sec and occasional sino-auricular block. The latest data indicate that the heart had increased in size and the auricular fibrillation persisted.

Patient 7 was examined elsewhere in September 1965. The heart was large; the ECG disclosed auricular fibrillation. This man was treated with digitalis until his sudden death in January 1966, aged 50. No postmortem was performed.

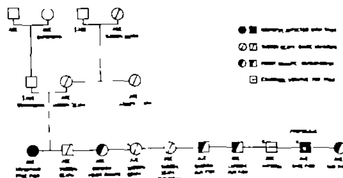


Fig. 8. Pedigree of described family with myocardial fibrosis.



Fig 9 Kidney of patient 1 A small kidney artery with arteritis. H-E, 35

Patient 8 46-year-old male, is still alive; he shows no cardiac symptoms but suffers from marked hypogonadism. W examined this man recently There were no cardiac symptoms.

Patient 9 is our case described in detail.

Patient 10 is 45-year-old female, still alive but suffering from severe angina pectoris; on examination auricular fibrillation and cardiomegaly were found.

DISCUSSION

The histories of the members of this family the clinical findings in patients, 3 6, 7 and 10 the postmortem findings in patient 1 and the propositus convinced us that this family is suffering from familial myocardial fibrosis

Differential diagnosis

The changes described should be differentiated from amyloidosis of the heart (by muscle biopsy (2) or rectal biopsy) and constrictive pericarditis, the latter is not a familial condition and can be quickly identified by the calcified pericardium. However it does give rise to the same haemodynamic phenomena as seen in myocardial fibrosis.

Similar haemodynamic changes can also be caused by endomyocardial fibrosis, as is observed in tropical countries (much less frequently in Europe). The cause of this disease may be a nutritional deficiency for the condition is common in underdeveloped countries. A diagnosis of myocardial fibrosis can be established with certainty only by myocardial biopsy but this is a rather dangerous procedure, of no therapeutic benefit to the patient. The findings of the ECG changes such as QS complexes in affected family and in young patients warrants careful consideration of the possibility of a degenerative myocardial process. Myocardial hypertrophy and fibrosis may also be found in patients with arachnodactyly (Marfan s syndrome) (1).



Fig 10 Heart of patient 1 Fibrosis of cardiac muscle. H-E. 3

Aetiology

Little is known about the causes of this condition, some authors think of some relation to Friedreich's ataxia (but in the latter the neurological changes predominate) and of progressive muscular dystrophy (affecting the skeletal muscles much more than the myocardium). Some authors mention the presence of glycogen or mucopolysaccharides in the affected myocardium. Exhaustive histochemical and electron-microscopic examination of the myocardium in such patients is advisable. This type of study may contribute to the detection of aetiological factors.

Heredity

In view of the deaths at an early age of the grandmother the mother and one of her sisters, it seems likely that the disease is transmitted as a dominant factor by the females.

A striking feature is that so many members of one generation were affected by what was probably the same disease; this has not been proved with certainty because postmortem data were available from only two members of the family It is not unreasonable to assume that the other early deaths were based on the same cause.

It would be interesting to make prospective study of the fourth generation (i.e. the children of the so markedly affected third generation) we intend to undertake such an investigation in due course and hope that it may contribute to a determination of the causal factors.

Therapy

Therapeutic efforts are hopeless in the long run, nearly all patients dying at an early age.

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VENOUS PRESSURE CHANGES DURING CONGESTION

Venous Pressure in the Great Saphenous Vein during Congestion with a Blood Pressure Cuff Proximal to the Knee

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Abstract. The pressure in the great saphenous vein at the medial malleolus increased to about 60% of cuff pressure of 60, 70 or 100 mm of mercury when 7 cm wide blood pressure cuff was placed at the upper edge of the patella or 10 cm more proximally. When that cuff was replaced by another, 16 cm wide, the venous pressure increased to about 90% of the cuff pressure. In all instances the venous pressure increased immediately after the application of the cuff pressure and reached constant level at between half minute and fiv minutes.

It is generally held that the pressure in the venous system distal to stasis produced by means of a blood pressure cuff is nearly equal to the pressure of the cuff as long as it is below the systolic arterial pressure (12, 16). This seems to be true of slim extremities such as the upper human extremity (14 18), and probably those of cats, dogs and rabbits. The conditions in the lower human extremity have been discussed in relation to venous occlusion plethysmography (3 5 11 17), two of the main questions being:

1 What are the relations between the values of the cuff pressure and the venous pressure distal to the cuff?

2. How rapidly is the venous outflow from the congested part of the limb reestablished after the application of the cuff pressure?

In order to answer these questions the following experiments were undertaken

MATERIAL AND METHOD

In eight males and tw females (6 patients ith cardiac disease, ith hypertension, and 2 normal persons) the pressure in the great saphenous vein near the medial malleolus as measured during congestion in the supine position by a percutaneously introduced catheter (Argyle Medicut, i.d. 0.90 mm) and Hansen manometer (9). More than 80 measurements ere made. The congestion was produced by two different blood pressure cuffs, 7 and 16 cm in idth, respectively. The cuff was placed either just proximal to the patella or 10 cm more proximal.

Cuff pressures of 60 70 and 100 mm of mercury ere used. In controlling the accuracy of the mercury manometer the cuff pressure values ere found to be the same, measured either by the Hansen manometer or the mercury manometer

RESULTS

The venous pressure began to increase immediately after the application of the cuff pressure and then reached a constant level after a certain time

The mean values and S.D.s are given in Table I. Results from a few experiments in which a cuff pressure of 100 mm of mercury were applied have been omitted since these results were analogous to the others.

The results tabulated are: the venous pressure when the constant level was reached (vp) this vp expressed as per cent of the cuff pressure (vp%), and the time in seconds that passed from the application of the cuff pressure till the constant level was reached (sec).

The mean value of the vp% resulting from the experiments with the 7 cm wide cuff amounts to 60.5 and that from the experiments with the 16 cm wide cuff to 89.3 the difference being significant on 1% level. The differences between the mean alues of the vp% resulting from the same cuff being placed at the proximal edge of the patella and 10 cm more proximally respectively are not significant on a 5% level. The differen-

Table I *Constant venous pressures obtained during congestion with two cuffs placed on two different levels of femur*

M V = mean value p = venous pressure when constant level reached, vp% = vp as per cent of the pplied cuff pressure, sec = time i seconds from application of cuff pressure until constant level reached

Distance of cuff to upper border of patella (cm)		0			0			10			10		
Cuff pressure (mmHg)		60			70			60			70		
Cuff width (cm)		p	vp%	sec	vp	vp	sec	vp	vp%	sec	vp	p	sec
7	M V	34.3	57 2	84 4	41 7	59 6	85.1	37 4	62.4	85 9	44 1	6 9	89.3
	S.D	4 0	6.8	35	4 4	6.6	37	6.2	9 0	48	6.5	9 6	42
16	M V	53.7	89 4	141.5	61.8	88.5	153.6	53.7	89 4	149 4	62.1	88.8	171.7
	S D	2.9	4 7	57	3.2	5.0	59	3.6	6 6	57	4 1	7 7	74

ces between the S.D of the vp% of the two different cuffs are significant on a 5% level only for the values resulting from the narrow cuff being placed 10 cm proximal to the upper edge of the patella (9 0 and 9 6) and the wide cuff being placed just proximal to patella (4 7 and 5 0).

If the mean values of the sec are converted to rate of increase, expressed as mm of mercury per 10 sec (Table II) the differences are small and not significant. The same holds for the differences tween the S.D s. However the rate of increase id vary a great deal from one person to another (from 2.15 to 7 94 mm of mercury per 10 sec) but was rather constant for the same subjects.

DISCUSSION

It is assumed on the basis of the present experiments that the vp, having reached a constant level exceeds the tissue pressure on the veins beneath the cuff— the effective cuff pressure — and so the venous outflow from the congested extremity is reestablished. This assumption was verified by a single direct catheterization of the femoral vein The tip of the catheter was placed beneath the middle of the cuff The pressure measured here was 1 mm of mercury below the vp measured in the great saphenous vein during the same congestion period.

Table II. *Rate of increase of venous pressure during congestion with two cuffs placed on two different levels of femur*

Cuff width (cm)	Distance of cuff to upper border of patella (cm)	Cuff pressure (mmHg)	Rat of increase (mmHg 10 sec)
7	0	60	0.41
7	0	70	0.49
7	10	60	0.43
7	10	70	0.49
16	0	60	0.38
16	0	70	0 40
16	10	60	0.37
16	10	70	0.36

The effective cuff pressure depends on the relation between the circumference of the extremity and the width of an otherwise proper cuff (1 13). Thus the mean value of all the vp% with the 16 cm wide cuff amounts to about 30% more than that of the 7 cm wide cuff As mentioned here the S.D of the vp% for the 7 cm wide cuff placed 10 cm proximal to the patella is significantly higher than that of the 16 cm wide cuff placed just above the patella. The reason for this is probably also the relation between the circumference of the extremity and the cuff width, in this case the wider cuff around the smaller circumference and vice versa.

The differences are of importance concerning occlusion plethysmography in which normally a narrow cuff is used (7). In experiments where capillary filtration is investigated, a certain measured volume increase of the lower extremity is set in relation to the capillary pressure (15). If this pressure is estimated on the basis of the cuff pressure, it is important to realize how much of the cuff pressure is transmitted to the deep veins ("the effective cuff pressure").

It is suggested that a cuff used in occlusion plethysmography if placed on the thigh, should be placed at the upper edge of the patella and, furthermore, that an estimation of capillary pressure is based on venous pressure. For investigations on arterial flow with occlusion plethysmography quite different aspects prevail (8).

The rate of increase of the superficial venous pressure is related to the filling of the vascular system distal to the cuff prior to the experiment, as, for instance, it took more time to reach the constant level when the subject was lying in a Trendelenburg position prior to and during the experiment. In addition, the blood flow rates through the different tissues of the limb are unequal, and the distensibilities are not necessarily the same (4). For these reasons too much attention should not be paid to the mean values or S.D.s of the rate of increase.

It should be taken into consideration that the venous pressure was measured only in a superficial vein. Therefore the pattern of the pressure increase in the whole venous system distal to the cuff cannot be known from the present experiment, nor does it show when the centripetal flow starts again after the application of the cuff pressure. However Conrad and Green (6) measured in dog paw both venous pressure and outflow during stasis, and found that the outflow began just before or when the constant level was reached.

Simultaneous measurements of the pressure in the deep and superficial veins in the standing position showed only a slight difference (2), or no difference at all (10 15). It is suggested that gravity in the erect position acts in a similar way as stasis produced by the cuff in the supine position—although the pressure values in the erect position are higher than in these experiments. Therefore it is believed that in the present experiments a constant level is also reached in the deep veins, amounting to nearly the same value.

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HISTAMINE STIMULATION OF GASTRIC INTRINSIC FACTOR SECRETION

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Abstract. The effect of histamine (subcutaneously or intravenously) on the gastric intrinsic factor (IF) secretion has been studied. The hourly IF output after subcutaneous histamine was correlated with the hourly outputs after intravenous histamine and with the hourly outputs after insulin and tetragastrin stimulation. It is concluded that the hourly IF output after 40 μg histamine diphosphate per kg body weight subcutaneously can be applied as physiologically relevant parameter of the IF secretory capacity

After the development of radioimmunoassay for gastric intrinsic factor (IF) (1 2) it soon became a convention to use the IF output for one hour after stimulation of the gastric secretion as parameter of the gastric IF secretory capacity A number of secretagogues have been employed. histamine, betazole, insulin, gastrin, pentagastrin, and tetragastrin (4 8, 9 11 14 15 16). By far the most commonly used has been histamine subcutaneously injected in a dose similar to that used in the augmented histamine test (10): 40 μg histamine diphosphate per kg body weight.

The purpose of the present study has been to investigate some aspects of the effect of histamine on the gastric IF secretion and to discuss whether the commonly used posthistamine hourly output as a parameter of physiological relevance.

MATERIAL AND METHODS

Gastric juice was aspirated as previously described (14) for one hour before and one or two hours after stimulation of the gastric secretion After stimulation gastric juice was aspirated in 15-min periods. From each period sample was taken for IF assay (14) From the IF concentration and the aspirated olume the IF secretion rate could be calculated in kilo-units (KU) per 15 min (or per hour). One kilo-unit of IF is the amount that binds one μg vitamin B (14). The coefficient of variation for the IF assay (from several determinations on 50 μl of standard human gastric juice, over several days) was 7 1%

With one exception (Table VI) the subjects who took part in the study were hospitalized patients with peptic ulcer dyspepsia. The acid secretion in these subjects was normal or high the maximal acid output at titration to pH 3.3 (4 5) ranged from 13 6 to 58 1 mEq h.

The effects of subcutaneous (40 μg kg body eight) and intravenous continuous (40 μg kg h) administration of histamine diphosphate on IF secretion were compared on separate days in nine subjects. These doses correspond to the doses necessary for maximal acid secretion (10, 12).

Four subjects had on separate days tw different doses of histamine diphosphate for stimulation (40 and 80 μg/kg subcutaneously)

The effect of histamine on IF secretion was compared to that of tetragastrin (11) and insulin (3) in 24 subjects on separate days the IF secretion after 40 μg histamine diphosphate kg subcutaneously was measured in all 24 subjects, in twelve the IF secretion after 20 μg tetragastrin/kg subcutaneously was measured, and in the remaining twelve subjects the IF secretion after 0 4 ternational unit of soluble insulin kg body weight intravenously

In five subjects duplicate determinations of the hourly IF secretion after stimulation with 40 μg histamine diphosphate kg subcutaneously were carried out on separate days In one normal subject seven determinations of this parameter were made.

The Student's *t*-test for pair differences was used for statistical analyses.

RESULTS

IF secretion as function of time after histamine stimulation

The effect of subcutaneous and intravenous histamine administration on the IF secretion is shown in Tables I and II.

When histamine was given subcutaneously the 15-min IF secretion rate increased significantly after stimulation from period 5–9 a gradual de cline in the IF secretion rate was observed, and

Table I. *IF secretion rate (KU/15 min) in 12 consecutive quarter-hourly periods (4 before and 8 after stimulation with 40 µg histamine diphosphate/kg body weight subcutaneously) in nine subjects*

N.S. = not significant ($p \geq 0.05$)

Subject no.	Basal secretion. Period 1	2	3	4	Stimulated secretion. Period 5	6	7	8	9	10	11	12
1	1.3	1.1	1.4	0.5	7.0	4.2	3.0	0.9	0.3	0.2	0.7	0.5
2	2.1	1.3	2.3	1.1	14.3	6.9	3.8	0.8	0.3	0.3	0.6	1.2
3	1.7	1.6	0.9	1.2	16.8	9.4	7.1	2.1	0.7	0.3	0.5	1.2
4	0.6	1.1	0.4	0.7	7.5	4.4	3.3	0.9	0.2	0.4	0.5	0.4
5	2.3	1.6	1.8	2.5	15.2	7.8	5.3	1.2	0.4	0.7	0.6	1.5
6	2.0	2.4	2.3	1.1	12.1	6.5	3.9	0.7	0.5	0.7	0.5	1.7
7	0.4	0.5	0.8	0.5	10.3	6.2	4.3	0.4	0.2	0.5	0.4	0.8
8	0.9	0.8	1.5	0.6	9.2	6.8	2.0	1.4	0.7	0.2	0.5	1.1
9	2.0	1.2	1.4	2.1	17.0	9.1	4.1	2.2	1.2	0.3	0.4	2.0
Mean	1.5	1.3	1.4	1.1	12.2	6.8	4.1	1.2	0.5	0.4	0.5	1.2
S.D	0.7	0.6	0.7	0.7	3.9	1.8	1.5	0.6	0.3	0.2	0.1	0.5
P-value, diff		N.S.	N.S.	N.S.	<0.001	<0.001	<0.001	<0.001	<0.001	N.S.	N.S.	<0.01

the IF secretion rates in the periods 9–11 were below the basal levels. From period 11 to 12 the IF secretion rate increased to basal levels.

During continuous intravenous histamine stimulation the IF secretion rate increased significantly from period 4 to 5. Hereafter the decline in the IF secretion rate was less steep than after subcutaneous histamine, and in the last stimulated hour (period 9–12) the IF secretion rate was at constant level higher than the basal values.

IF hourly secretion after histamine

From the individual values in Tables I and II the hourly IF secretion rates could be calculated for comparison.

After subcutaneous histamine the basal hour secretion rate and the secretion rate in the first hour after stimulation were both higher than the secretion rate in the second hour after stimulation ($P < 0.001$). The secretion rate in the first stimulated hour was higher than the basal secretion rate ($P < 0.001$).

When histamine was given by intravenous constant infusion the first and second stimulated hourly secretion rates were both higher than the basal secretion rate ($P < 0.001$). The secretion rate in the first was higher than in the second stimulated hour ($P < 0.001$).

The following correlations were derived from the data. The IF secretion rate in the first hour

Table II. *IF secretion rate (KU/15 min) in 12 consecutive quarter-hourly periods (4 before and 8 during stimulation with 40 µg histamine diphosphate/kg body weight/h intravenously) in nine subjects*

N.S. = not significant ($p \geq 0.05$)

Subject no.	Basal secretion. Period 1	2	3	4	Stimulated secretion. Period 5	6	7	8	9	10	11	12
1	1.4	2.1	0.5	0.7	9.1	4.9	4.1	2.1	1.7	1.9	1.9	1.7
2	1.8	0.7	1.2	0.4	9.6	6.5	4.9	2.9	3.1	2.7	3.0	3.5
3	1.5	1.4	2.7	1.6	15.7	9.5	7.2	3.8	3.7	3.0	3.3	3.5
4	0.8	1.7	0.3	0.7	7.7	5.1	3.5	2.4	2.3	2.5	2.1	2.1
5	1.0	0.8	1.2	0.9	16.1	10.9	8.1	5.2	4.1	4.3	4.7	4.5
6	1.2	1.8	0.6	1.5	13.9	9.7	8.2	3.6	3.7	3.5	2.7	3.1
7	0.5	0.4	1.3	1.2	15.0	10.2	7.3	4.1	3.5	3.3	2.8	2.7
8	1.4	1.0	0.7	1.4	11.6	5.1	3.8	2.3	2.6	2.2	2.9	2.7
9	1.8	1.3	0.4	0.5	19.6	11.2	8.9	5.1	4.4	4.7	4.5	4.9
Mean	1.3	1.2	1.0	1.0	13.1	8.1	6..	3.5	3.2	3.1	3.1	3.2
S.D	0.5	0.6	0.8	0.5	3.9	2.7	2.1	1.2	0.9	0.9	1.0	1.1
P-value, diff.		N.S.	N.S.	N.S.	0.001	<0.001	<0.001	<0.001	N.S.	N.S.	N.S.	N.S.

Table III. *IF secretion rate (KU/h) after 40 and 80 µg histamine diphosphate/kg body weight subcutaneously on separate days in four subjects*

Subject no.	IF secretion rate (KU/h) 40 µg	80 µg
10	29.1	27.9
11	30.5	34.7
12	27.4	30.5
13	47.5	41.8

after subcutaneous histamine was correlated with the secretion rate in the first ($r = 0.77$, $P < 0.05$) and the second hour ($= 0.83$, $P < 0.01$) during intravenous histamine. The secretion rate in the first hour was correlated with the secretion rate in the second hour during intravenous histamine ($= 0.90$, $P < 0.01$).

Dose of histamine subcutaneously

In four subjects the IF secretion rates (kU/h) after 40 and 80 µg of histamine diphosphate/kg subcutaneously were of the same order (Table III).

Comparative effects of histamine, tetragastrin and insulin

The IF secretion rate after histamine in twelve subjects (Table IV) was correlated to the IF secretion rate after tetragastrin, and the mean difference was not significantly different from zero (t-test for pair differences).

When one subject was excluded (in whom the IF secretion rate after insulin was considerably higher than after histamine) the secretion rate after histamine was correlated with the secretion rate after insulin (Table IV). The mean difference of the two secretion rates differed significantly from zero (t-test for pair differences).

Reproducibility of hourly IF secretion after subcutaneous histamine

The results of duplicate determinations of the IF secretion rate after subcutaneous histamine (kU/h) are given in Table V and the results of repeated investigations in the same subject in Table VI. The coefficient of variation was considerably higher for the basal IF secretion rate than for the histamine-stimulated secretion rate.

DISCUSSION

The gastric secretion is usually divided into three phases: a cephalic phase, a gastric phase, and an intestinal phase. Although this division is useful for analyzing physiological mechanisms, it is artificial because normally these phases overlap and interact (7).

For clinical testing of gastric *acid* secretion, gastrin or any synthetic peptide related to gastrin has no theoretical advantage over histamine (5, 7). The responses to all these agents are highly correlated, and all yield a reliable index of the acid secretory capacity. The same apparently holds true for the IF secretion, since the IF outputs after gastrin, pentagastrin, tetragastrin, insulin, betazole and histamine are similar (3, 8, 9, 11, 14, 15, 16, and Table IV).

The physiological role of histamine in gastric secretion is unresolved (7) but it is recognized (13) that the acid secretion after the intake of solid food is of the same order as that seen during

Table IV. *Comparative effects on IF hourly secretion (KU/h) after stimulation with histamine (40 µg histamine diphosphate/kg body-weight subcutaneously), tetragastrin (20 µg/kg body weight subcutaneously), and insulin (0.4 international unit of soluble insulin/kg body weight intravenously)*

N.S. not significant ($p = \geq 0.05$)

No. of subjects studied	Stimulation	Mean	S.D.	Mean difference	Significance of difference	Coefficient of correlation	Significance of correlation
12	Histamine	29.5	6.8	3.1	N.S.	0.68	$p < 0.05$
12	Tetragastrin	32.7	8.3				
12	Histamine	28.1	8.6	3.3	N.S.	−0.32	N.S.
12	Insulin	24.8	8.9				
11	Histamine	29.5	7.3	5.5	p 0.05	−0.63	$p < 0.05$
11	Insulin	24.1	8.9				

Table V *Duplicate determination of the IF secretion rate (KU/h in one basal hour and in one hour after stimulation with 40 µg histamine diphosphate/kg body weight subcutaneously) on separate days in five subjects*

Subject no	Intrinsic factor secretion rate (KU/h)			
	First examination		Second examination	
	Basal h	Stim. h	Basal h	Stim. h
14	2.7	21 2	1 2	26.7
15	6	30 4	4 9	24.2
16	2.1	19 0	1.3	14 5
17	2 5	29 5	4 7	33.4
18	7 4	32 8	3.2	38 9

intravenous maximal histamine infusion (40 µg histamine diphosphate kg h).

Previously it was suggested that food stimulates the gastric IF secretion (6, 17) By means of IF radioimmunoassay it was recently shown that maximal histamine stimulation and the oral administration of a protein hydrolysate both resulted in a marked increase of the IF hourly output (18). The IF output ollowing histamine seems e a parameter f physiological relevance. the intake of food the IF secretion is probregulated via the agal nerves and the re- of endogenous gastrin. It can be assumed, owever for the reasons above that whether or not histamine is in ol ed as a final common mediator for gastric secretion the secreted IF amounts and the IF secretory pattern after the intake of food closely mimic the secretion during maximal intravenous histamine infusion (19 and Table II).

Table VI *Repeated investigations on IF secretion rate (KU/h in one basal hour and in one hour after stimulation with 40 µg histamine diphosphate/kg body weight subcutaneously) on separate days in one normal subject*

N of investigation	IF secretion rate (KU h)	
	Basal h	Stim h
1st	4 4	12 4
2nd	3 0	13 2
3rd	2.1	14 5
4th	2.9	15 8
5th	1 3	10 8
6th	3.1	12 8
7th	2.7	17 2
Mean	2.8	13 8
S.D	1.0	2 2
Coefficient of variation (%)	34	15

It is debatable which expression should be used as parameter of the IF secretory capacity In all probability the secretion in the first hour after stimulation is mostly due to release of an IF depot in the gastric mucosa (3 16, 19). From a physiological viewpoint it seems reasonable that the IF parameter should include the depot secretion The present study shows that the IF outputs in the first hour after subcutaneous histamine injection and in the first and second hours during intravenous histamine infusion all are correlated. The dose of histamine used in the augmented histamine test seems maximal for histamine stimulation of the IF secretion (19 and Table III), and with this test the IF hourly output is fairly reproducible (Tables V and IV). It can thus be concluded that the IF hourly output after the subcutaneous injection of histamine diphosphate (40 µg/kg body weight) can be applied as a physiologically relevant parameter of the gastric IF secretory capacity If in the future one of the synthetic peptides related to gastrin (pentagastrin, tetragastrin) replaces histamine for clinical gastric secretion tests, the effect on IF secretion of these agents will be directly comparable to the effect of histamine (9 12, 15)

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THE INTRINSIC FACTOR SECRETION IN LOCALIZED GASTRIC DISORDERS

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Abstract. By means of radioimmunoassay the gastric secretion of intrinsic factor (IF) has been measured in 228 subjects, comprising 65 duodenal ulcer patients, 50 patients with gastric ulcer, 25 patients with gastric carcinoma, 35 patients with Addisonian pernicious anaemia, and 53 control subjects. The IF secretion in kilo-units (KU) in the first hour after augmented histamine stimulation was taken as parameter of the gastric IF secretory capacity. One KU = the amount of IF that binds one ng vitamin B_{12}. Mean ± S.D. (in KU/h) was (number of subjects in parentheses): male controls (25) 17.4 ± 6.8, female controls (28) 13.7 ± 5.1, males, duodenal ulcer (54) 28.2 ± 8.7, females, duodenal ulcer (9) 18.0 ± 5.–, males, corpus ulcer (13) 11.2 ± 4.8, females, corpus ulcer (17) 12.7 ± 5.5, males, prepyloric ulcer (13) 16.8 ± 5.0, females, prepyloric ulcer (7) 18.6 ± 5.0. The IF secretion in the 25 gastric cancer patients ranged from 0.1 to 28.2 KU/h, and was below 8.5 in 22 patients. The IF secretion in the 35 patients with pernicious anaemia was in all cases below 0.2 KU/h. As the daily need for IF is 2–4 KU, it is concluded that in subjects with normal gastric mucosa the IF secretory capacity is abundant in relation to the need for IF.

By means of the augmented histamine test (16) several authors have under standardized conditions investigated the gastric acid secretion in healthy subjects and in patients with various gastric disorders (6, 7, 8, 11, 13, 14, 17, 19). These investigations have shown that on the average normal subjects secrete about 20 mEq hydrochloric acid per hour, that duodenal ulcer patients secrete approximately twice the normal amount, that the secretion in gastric ulcer patients is somewhat lower than normal, and that patients with gastric carcinoma often have a low acid secretion with a high incidence of achlorhydria.

Physiologically the gastric secretion is essential only for the absorption of vitamin B_{12}. This absorption is normally mediated via the gastric intrinsic factor (IF), a glycoprotein with strong affinity for vitamin B_{12}. Until a few years ago IF could be measured only by semiquantitative time consuming methods. The development of a radioimmunoassay for IF (1, 3) has made possible quantitative IF measurement in vitro. In this field most attention has hitherto been paid to the elucidation of the problem whether this new method enables the clinician to make a more rapid and accurate diagnosis of pernicious anaemia (4, 15, 24).

The aim of the present study has been to present a systematic study of the IF secretion in localized gastric diseases compared with the secretion in healthy subjects and in patients with pernicious anaemia.

METHODS

In the study of the IF secretion in each subject the following procedure was employed. After an overnight fast an augmented histamine test (16) was performed. A radioopaque Rüsch nasogastric tube no. 16–18 was optimally sited under X-ray control (so that its tip lay over the vertebral column in the midline). The stomach was emptied and its content discarded. Gastric juice was then collected for two hours: the basal secretion was collected for one hour and the histamine-stimulated secretion for one hour in four 15-min periods. The gastric secretion was stimulated with 40 μg histamine acid phosphate/kg bodyweight injected subcutaneously. Half an hour before the histamine injection 50–100 mg mepyramine maleate was given intramuscularly. Continuous aspiration of gastric juice was achieved by using an electric pump (constant negative pressure 50 mmHg), frequently interrupted by manual suction and injection of air to ensure patency of the tube. Collection of gastric juice was done either by the author or by well-experienced assistants in a laboratory specialized in the study of gastric secretion.

Secretory volume and acid secretion were determined in the basal and the four activated portions. Hydrochloric acid was determined electrometrically with pH measure-

Table I *Values (mean and S.D.) for age and secretory hourly volume (ml/basal and stimulated h) in 228 subjects studied*

UCV = ulcer in the body of the stomach. N = control subjects. UD = duodenal ulcer. UPP = prepyloric ulcer. CV = gastric carcinoma. PA = pernicious anaemia. Ac = free acid. Hyp = hypochlorhydria. Achl = achlorhydria

No. of subjects		Age (y) Mean	Age (y) S.D	Basal volume (ml/h) Mean	Basal volume (ml/h) S.D	Stim. volume (ml/h) Mean	Stim. volume (ml/h) S.D
25	N (♂)	42	18	75	36	257	46
28	N (♀)	38	13	80	36	199	45
56	UD (♂)	47	13	142	57	390	75
9	UD (♀)	49	7	111	37	284	73
13	UCV (♂)	55	8	80	40	180	70
17	UCV (♀)	61	16	73	31	184	59
13	UPP (♂)	57	9	76	27	252	75
7	UPP (♀)	50	8	87	59	244	61
11	CV (♂ + ♀), Ac	68	13	71	33	162	88
4	CV (♂ + ♀), Hyp	74	8	37	16	57	9
10	CV (♂ + ♀), Achl.	75	7	47	37	32	4
11	PA (♂)	7?	10	26	18	23	15
24	PA (♀)	65	11	21	12	22	15

ment and automatic titration with 0.1 N NaOH to pH 3.3. The criteria for achlorhydria and hypochlorhydria were those of Callender et al. (8) and Christiansen (10).

In the present study the following expressions are used. Basal volume (ml/h) = gastric juice aspirated in the basal hour. Stimulated volume (ml/h) = gastric juice aspirated in the histamine-stimulated hour. Maximal stimulated acid concentration (mEq/l) = the highest acid concentration at titration to pH 3.3 in the four stimulated 15-min portions. Stimulated acid output (mEq/h) = the sum of four stimulated acid concentrations multiplied by respective 15-min volume. This sum is the "maximal output" as used by several authors as parameter of the maximal gastric acid secretory capacity (12).

From the basal and from each of the four stimulated portions a sample of gastric juice [illegible] taken for IF assay. The sample was immediately neutralized with 1 N NaOH to pH 7–8 and [illegible] 20°C until assay. IF was determined rad[illegible]ically (1, 3, 4). The detailed procedure of the [illegible] described elsewhere (23). With this assay the [illegible] of [illegible] sample of gastric juice is expressed as [illegible] bound to IF. It has become com[illegible] IF activity in units, one unit being the IF binding of one ng of radioactive cyanoco[illegible]. [illegible] measure is appropriate in describing IF [illegible] (units/ml). In describing the hourly IF [illegible] often useful to express these in kilo-units (21 [illegible] kilo-unit of IF is the amount of IF that specifically binds one μg of radioactive cyanocobalamin.

The following expressions are used. Basal IF output (units/h or kilo-units/h) = the basal IF concentration (after correction for dilution with 1 N NaOH) multiplied by the basal volume. Stimulated IF output (units/h or kilo-units/h) = the sum of the four stimulated IF concentrations (after correction for dilution with 1 N NaOH), each multiplied by the respective 15-min volume. The mean stimulated IF concentration (units/ml) = the stimulated IF output divided by the stimulated volume.

MATERIAL

The gastric secretion of IF was studied in 228 subjects. They were: 53 control persons (25 males, 28 females), 65 patients with chronic duodenal ulcer (56 males, 9 females), 50 patients with gastric ulcer (26 males, 4 females), 25 patients with gastric carcinoma (18 males, 7 females), and 35 patients with Addisonian pernicious anaemia (11 males, 24 females).

Of the 53 control persons 28 were healthy volunteers and 25 were patients with minor diseases (usually inguinal hernias) unlikely to influence gastric secretion. None of the control persons had ever had dyspepsia suggestive of peptic ulcer or gallstones, and they were all in good general health.

The 65 patients with chronic duodenal ulceration all had typical clinical and radiological features. They were selected at random in the course of 28 months. In 63 of the patients (56 males, 7 females) pyloroplasty and total vagotomy were done shortly after the histamine test, and the diagnosis of duodenal ulcer was confirmed in all cases.

The 50 patients with gastric ulcer were selected at random in the course of 27 months. All patients had typical clinical and radiological features. The diagnosis was later confirmed at operation in 32 patients. In the remaining patients the ulcer niche disappeared or diminished after medical treatment. According to the localization of the ulcer niche, the patients could be divided into 20 patients with prepyloric ulcer (13 males, 7 females) and 30 patients with ulcer in the body of the stomach (13 males, 17 females).

The 25 patients with gastric carcinoma were selected at random in the course of 15 months. An operation was later done in 24 patients and the diagnosis confirmed in all cases. The diagnosis in the remaining inoperable patient was confirmed radiologically and by gastroscopy. The tumour was mainly situated in the cardiac region in four patients, in the pyloric region in seven patients,

Table II. *Values (mean and S.D.) for basal acid output (mEq/h), maximal acid output (mEq/h), and maximal acidity (mEq/l) in 179 acid-secretors*

N control subjects. UD = duodenal ulcer UVC = ulcer in the body of the stomach. UPP = prepyloric ulcer CV = gastric carcinoma. Ac = free acid

No. of subjects		Basal acid output (mEq/h) Mean	S.D.	Maximal acid output (mEq/h) Mean	S.D.	Maximal acidity (mEq/l) Mean	S.D.
25	N (♂)	1.8	2.1	22.8	6.2	100	17
28	N (♀)	1.8	1.6	17.3	5.9	99	20
56	UD (♂)	6.8	5.5	39.9	10.4	112	15
9	UD (♀)	4.4	4.9	26.0	9.4	104	13
13	UCV (♂)	0.8	1.4	10.7	6.9	69	19
17	UCV (♀)	1.3	1.6	12.1	6.5	75	16
13	UPP (♂)	1.3	1.3	19.7	8.8	88	18
7	UPP (♀)	2.4	2.6	20.3	6.6	99	12
11	CV (♂+♀), Ac	1.1	1.5	8.9	9.5	61	38

in the body of the stomach in five patients. In the remaining nine patients extensive tumours were found. Eleven of the patients had an acid gastric juice after histamine stimulation, four had hypochlorhydria, and ten achlorhydria.

All the 35 patients with Addisonian pernicious anaemia had achlorhydria, and they had all, at the time of the investigation, responded typically to treatment with vitamin B_{12} for megaloblastic anaemia. None of the patients had symptoms suggestive of intestinal disease, and in the majority of the patients serum-vitamin B_{12} had been determined and urinary excretion test with radioactive vitamin B had been made, which in all cases showed values typical of pernicious anaemia. In nine of the patients the diagnosis was newly made, while the remaining 26 patients had had the disease for 1 to 18 years (mean 6 years).

For characterization of the different groups the values for age and volume secretion are given in Table I, and for the 179 patients with an acid gastric juice after stimulation the values for acid secretion are summarized in Table II (53 controls, 115 ulcer patients, and 11 patients with gastric carcinoma).

In the text and the tables the following abbreviations are used: N = control subjects, UD = duodenal ulcer UV = gastric ulcer (prepyloric + body localization), PPU = prepyloric ulcer UCV = ulcer in the body of the stomach, CV = gastric carcinoma, PA = pernicious anaemia, IF = intrinsic factor KU = kilo-units.

The results are analysed by standard statistical methods if not otherwise stated.

RESULTS

Basal IF secretion

The distributions and the values for basal IF secretion are given in Table III. A characteristic

Table III *Distributions of basal IF secretion (KU/h) in the 228 subjects studied (mean, median, S.D. and range also given)*

N control subjects. UD = duodenal ulcer UCV = ulcer in the body of the stomach. UPP prepyloric ulcer CV = gastric carcinoma. PA = pernicious anaemia *Ac = free acid. Hyp = hypochlorhydria. Achl achlorhydria*

Subjects	Class intervals (KU/h) 0.0–0.5	0.6–1.0	1.1–2.0	2.1–3.0	3.1–4.0	4.1–6.0	6.1–8.0	8.0	Total no.	Mean	Median	S.D.	Range
N (♂)	3	1	5	4	5	4	3	0	25	3.0	2.9	1.9	0.1–7.1
N (♀)	1	1	8	8	6	3	1	0	28	2.7	2.5	1.3	0.4–6.2
UD (♂)	2	0	1	4	7	11	8	23	56	7.2	7.3	4.0	0.3–20.7
UD (♀)	0	0	1	2	2	0	2	2	9	5.5	4.0	3.7	1.8–11.8
UCV (♂)	1	1	2	5	2	2	0	0	13	2.5	2.5	1.3	0.3–5.2
UCV (♀)	0	0	5	5	4	3	0	0	17	2.9	2.7	1.1	1.3–4.8
UPP (♂)	0	0	4	3	5	1	0	0	13	2.8	2.9	0.9	1.6–4.1
UPP (♀)	0	1	1	2	1	2	0	0	7	2.9	2.9	1.5	0.9–5.0
CV (♂ ♀), Ac	2	3	4	0	1	0	1	0	11	1.7	1.2	1.8	0.1–6.3
CV (♂ ♀), Hyp	2	0	1	0	1	0	0	0	4	1.2	0.8	1.3	0.1–3.1
CV (♂ ♀), Achl	9	1	0	0	0	0	0	0	10	0.2	0.1	0.3	0.0–0.8
PA (♂)	35	0	0	0	0	0	0	0	35				0.0–0.1

Table IV *Distributions of stimulated IF secretion (KU/h) in 228 subjects studied*

N = control subjects. UD = duodenal ulcer. UCV = ulcer in the body of the stomach. UPP = prepyloric ulcer. CV = gastric carcinoma. PA = pernicious anaemia. Ac = free acid. Hyp = hypochlorhydria. Achl = achlorhydria

Subjects	0.0–0.2	0.3–3.9	4.0–7.9	8.0–11.9	12.0–15.9	16.0–19.9	20.0–23.9	24.0–27.9	28.0–31.9	32.0–35.9	36.0–39.9	40.0–43.9	44.0–47.9	Total no.
	Class interval (KU/h)													
N (♂)	0	0	2	5	3	7	4			0	0	0	0	25
N (♀)	0	0	3	8	9	4	3	1	0	0	0	0	0	28
UD (♂)	0	0	0	0	6	4	8	12	8	8	3	5	1	56
UD (♀)	*0*	*0*	*0*	*1*	*1*	*5*	*1*	*1*	*0*	*0*	*0*	*0*	*0*	*9*
UCV (♂)	0	0	4	3	3	3	0	0	0	0	0	0	0	13
UCV (♀)	0		1	4	5	4	0	1	0	0	0	0	0	17
UPP (♂)	0	0	0	3	4	2	3	1	0	0	0	0	0	13
UPP (♀)	0	0	0	1	1	1	4	0	0	0	0	0	0	7
CV (♂+♀), Ac	0	3	4	1	1	0	1	0	1	0	0	0	0	11
CV (♂+♀), Hyp	0	3	1	0	0	0	0	0	0	0	0	0	0	4
CV (♂+♀), Achl	3	7	0	0	0	0	0	0	0	0	0	0	0	10
PA (♂+♀)	35	0	0	0	0	0	0	0	0	0	0	0	0	35

feature of the basal secretion was a high coefficient of variation. The mean IF secretion in male duodenal ulcer patients was significantly higher ($p < 0.01$ Wilcoxon rank sum test) than the secretions in male subjects of the N, UCV and UPP groups. The mean IF secretion in female duodenal ulcer patients was significantly higher ($p < 0.05$) than mean secretion in normal females. The basal secretion in the 35 PA patients was in all cases ow 0.1 KU/h. Of the 179 acid-secretors nine had a basal IF secretion between 0.1 and 0.5 KU/h.

Stimulated IF secretion

The distributions of the stimulated IF secretion are given in Table IV. For all 35 PA patients the IF secretion was below 0... KU/h (maximal value 190 U/h). Three achlorhydric CV patients had an IF secretion in the PA range. The mean values, standard deviations, and the ranges for the stimulated IF secretion in the 193 subjects without PA are given in Table V. For most stimulated hourly outputs two means were compared by using the Student's *t*-test.

Males secreted significantly more IF than fe

Table V *Values for stimulated IF secretion in KU/h (mean, S.D., range) and average stimulated IF concentration in U/ml gastric juice (mean and S.D.) in 193 subjects without pernicious anaemia*

N = control subjects. UD = duodenal ulcer. UCV = ulcer in the body of the stomach. UPP = prepyloric ulcer. CV = gastric carcinoma. Ac = free acid. Hyp = hypochlorhydria. Achl = achlorhydria

No. of subjects		Stimulated IF secretion (KU/h) Mean	S.D.	Range	Stimulated IF concentration (U/ml) Mean	S.D.
25	N (♂)	1 4	6.8	6.3–30..	66	20
28	N (♀)	13 7	5 1	4 9–25 0	61	20
56	UD (♂)	23	8 7	1..1–47 5	7?	18
9	UD (♀)	18 0	5.2	14 8–39 7	64	15
13	UCV (♂)	11..	4 8	4 6–19 3	63	16
17	UCV (♀)	1..7	5.5	3 1 4.2	69	19
13	UPP (♂)	16.8	5.0	9 8– 4 5	68	1
7	UPP (♀)	18.6	5 0	10 7 22.8	76	13
11	CV (♂+♀) Ac	9 7	8 4	1 4– 8..	58	?6
4	CV (♂+♀) Hyp	..6	..4	1 1 6.2	43	35
10	CV (♂+♀) Achl	0 8	0 7	0 1 ..	37	14

males in the N group ($p < 0.05$) and in the UD group ($p < 0.001$), while no sex differences were found in the UV subgroups.

For males the secretion in the N group was significantly lower than in the UD group ($p < 0.001$), significantly higher than in the UCV group ($p < 0.01$), and not significantly different from the secretion in the UPP group.

For females the secretion in the N group was lower than in the UD group ($p < 0.05$) and the UPP group ($p < 0.05$) and not significantly different from the secretion in the UCV group.

UD males secreted more than male UPP patients ($p < 0.001$) and more than male UCV patients ($p < 0.001$). Female UD patients secreted more than female UCV patients ($p < 0.05$), while the secretions in the female UD and UPP groups were of the same order

For both males ($p < 0.01$) and females ($p < 0.05$) the secretion in the UPP group was significantly higher than in the UCV subgroup of gastric ulcer

The mean secretion for the whole UV group (50 subjects) was significantly higher than the values for acid secreting CV patients and all CV patients ($p < 0.02$ and $p < 0.01$ Wilcoxon rank sum test). Twenty-two of the 25 CV patients secreted from 0 1–8.3 KU/h, while only seven of the 50 UV patients secreted less than 8.3 KU/h. Three of the four CV patients with cardiac tumour localization secreted acid and had IF secretions of 28.0, 21 1 and 8.3 KU/h. On the average CV patients with cardiac or pyloric tumour localization (11 patients, seven of whom secreted acid) had a higher IF secretion than the group with extensive tumours or tumours in the body of the stomach (14 patients, four of whom secreted acid), but the difference was not significant (means 7 6 and _9 KU/h, $0.05 < p < 0.1$ Wilcoxon rank sum test).

Correlations

For the UPP females a significant correlation between age and stimulated IF output was found ($= -0.75$ $p < 0.01$), while there was no significant correlation between age and stimulated IF output in the control persons or the other ulcer subgroups. The maximal acid output was correlated with the stimulated IF output in the control subjects ($= 0.65$ $p < 0.001$), in the UD patients

Table VI *Published reports on gastric IF secretion (KU/stimulated h) in control subjects, duodenal ulcer patients gastric ulcer patients and gastric carcinoma patients*

UD – duodenal ulcer UV – gastric ulcer CV – gastric carcinoma

	Year	No. of subjects studied	IF secretion (KU/h) Mean	Range
Control subjects				
Ardeman et al.	1964	9	8 9	2.2–18.3
Rodbro et al.	1965	15	16 2	5 4–25.0
Shearman et al.	1967	10	14.8	6 0–31.8
Wangel et al.	1968	12	13.8	5.0–24.0
Chanarin	1968	37 (♂)	9 1	
Chanarin	1968	32 (♀)	5.8	
Strickland et al.	1969	6	13 7	8.5–22.9
Present study		25 (♂)	17 4	6 3–30 2
Present study		28 (♀)	13 7	4 9–25.0
UD patients				
Ardeman et al.	1964	14	8 6	2.7–14.0
Rodbro et al.	1965	24	23.2	12 4–40.5
Adams et al.	1967	15	18 0	6 2–45.0
Shearman et al	1967	9	23.0	10.9–32.9
Wangel et al.	1968	7	21.8	12.5–35.0
Present study		56 (♂)	28.2	12.1–47.5
Present study		9 ( )	18 0	9 2 27 4
UV patients				
Rodbro et al.	1965	17	14 1	
Shearman et al	1967	11	12.8	6.0–23 1
Present study		50	14.2	3.1 24 5
CV patients				
Rodbro et al.	1965	7	1 7	
Shearman et al.	1967	22	3 2	0.1 9 0
Present study		25		0.1–28.2

($r = 0.71$ $p < 0.001$) and in the UV patients ($r = 0.81$ $p < 0.001$).

DISCUSSION

In most recent studies on the gastric secretion of IF the radioimmunologically measured IF output in the first 60 min after the subcutaneous injection of 40 μg histamine acid phosphate per kg body weight has been taken as parameter of the gastric secretory capacity This parameter though arbitrary is probably of physiological relevance (22). Its wide acceptance has made possible direct comparison of results from one worker to those from another

The amounts of IF found under standardized conditions in a group of subjects is mainly determined by three variables. 1) the assay for measure-

Congress Announcements

The Second National Congress of Gastroenterology will be held in Bucharest, September 9 to 11 1971

Topics

1 Nosological, diagnostical and therapeutical controversies in gastroenterology
2. Nonspecific chronic enteropathies.
3 Hepatic immunopathology

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FINE NEEDLE ASPIRATION BIOPSY FOR CYTODIAGNOSIS OF MALIGNANT TUMOUR IN THE LIVER

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Abstract. The accuracy of fine-needle aspiration biopsy and cytodiagnosis in the detection of intrahepatic malignant tumours is evaluated. In material of 1 748 biopsies, performed on varying and very wide indications, intrahepatic malignancy was diagnosed in 57 patients. Later in 39 of these, cancer was histologically proved. There was no false positive cytodiagnosis of cancer Fine-needle puncture detected about the same percentage of tumours as did angiography and scintigraphy The method proved safe, simple and reliable for diagnosing intrahepatic cancer It can be used either as an independent screening method for the detection of liver tumours, or for cytological analysis of localized foci detected by palpation, angiography or scintigraphy It is not invalidated by the hazards reported for conventional punch-needle biopsy when used in tumour-suspected cases.

Cancer growth in the liver is still too often an unexpected finding at laparotomy or autopsy There is thus a great need for simple and reliable methods of detecting its presence as early as possible When it is suggested by case history bedside observations, and biochemical findings, *angi*ography or scintigraphy may bring the clinical diagnosis close to certainty: this, however is never definite until the presence of malignant tumour cells has been demonstrated.

Biopsy is thus necessary for a diagnosis, and apparently the ideal method of obtaining decisive specimens would be directed biopsy during laparotomy or laparoscopy These are major interventions to be used only exceptionally There is still dispute in the literature about the value of conventional percutaneous biopsy (punch technique) for this purpose. Fenster and Klatskin (3) report a detection rate of up to 92 % of malignant liver tumours, whereas in another study blind liver puncture in cadavers revealed malignant tumour in only 70 % of the cases where no clinical evidences of liver tumour had been present during lif (7). Evidently the yield of *blind* percutaneous liver puncture must depend directly on the degree of tumour spread at the time of puncture A high incidence of complications made Fisher and Faloon (4) doubt the justification of using per cutaneous puncture (Vim Silverman technique) for the diagnosis of liver tumours.

To be well adapted for this purpose the method of liver puncture should obviously be innocuous enough to be justified on wide indications. An extensive experience with *fine-needle liver biopsy* for different purposes has convinced the uthor that this method is sufficiently free of hazards to be a useful tool in the clinical diagnosis of liver tumours. The scope of the present pap r is to analyse in retrospect the usefulness and reliability of this method in the diagnosis of liver tumours. The results of angiography and scintigraphy are compared with those obtained by cytodiagnosis.

METHODS

Aspiration biopsy was performed with needle, 0.6–0.7 mm diameter usually without previous anaesthesia, through an intercostal space in the mid-axillary line. When the liver was considerably enlarged, the biopsy was performed transabdominally preferably from palpable liver nodules. Two biopsies from different parts of the liver were made in 23 patients. Usually 2–6 smears were prepared from the aspirate They were stained, like ordinary blood smears, with May-Grünwald-Giemsa. The biopsy technique is described in detail by Söderström (8).

The following biochemical tests of liver function were generally used serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum bilirubin, serum alkaline phosphatase, and bromsulphalein retention test (BSP). Bromsulphalein retention as measured 30 min after an intravenous injection of 5 mg kg body weight. Normal values are shown in Figs. 5 6 and 7

Scintigraphy of the liver was performed with a rectilinear scanner. It was started 30–60 min after intravenous injection of 100 μCi colloidal Au (particle size 50–300 Å). The scintigrams were examined without knowledge of clinical and laboratory data by Dr E. Cederquist, Department of Radiotherapy, University Hospital, Lund.

Hepatic angiography was performed by selective catheterization of the hepatic artery or coeliac artery and in some patients also of the superior mesenteric artery (1). The angiograms were revised without access to clinical data by Dr E. Boijsen, Department of Diagnostic Radiology, University Hospital, Lund.

MATERIAL

A consecutive series of 1 748 fine-needle liver biopsies performed at the Cytodiagnostic Unit of the Department of Internal Medicine, University Hospital, Lund, in the years 1964–1967 were analysed retrospectively. Re-ex amination *of the clinical records gave histological diagnosis* of intrahepatic cancer in 50 patients. Their average age was 6. years; 65% were men. The presence of intrahepatic cancer in 45 cases was proved at autopsy within one year (mean 2 months) after the fine-needle puncture. In further 24 patients intrahepatic cancer in retrospect seemed highly probable because of the clinical findings and the course of the disease. These patients died out of the hospital, and no autopsy was performed.

It is local policy to perform liver puncture on very wide indications, and fine-needle biopsy is often used as a primary method to secure diagnosis of malignant tumour even when this is fairly evident from the bedside observations. It was possible to define specific group f patients in whom evidence of intrahepatic cancer appeared clinically convincing by the palpatory finding of an enlarged nodulated liver in addition to general symptoms suggestive of malignant tumour. At the time of liver puncture, cancer in the liver was *strongly suspected* in 44 patients. In numerous patients the possibility of intrahepatic cancer had often to be considered among other diagnostic issues, but it proved impossible to define *group* where intrahepatic cancer appeared slightly suspect on clinical grounds.

Scintigraphy as performed in 4 patients and angiography in 31 who later proved to have intrahepatic cancer.

The normal subjects referred to in this paper (Figs. 5, 6 and 7) constitute control material of previous study in which the principles of selection were presented (6).

Complications f fine-needle puncture

No complications of biopsy were encountered in this series. None of the patients showed evidence of intraperitoneal bleeding, peritonitis, or tumour spread following puncture.

RESULTS

Cytodiagnosis

In the entire material of 1 748 biopsies a cytodiagnosis of cancer was made in 57 patients (Table I).

Cytodiagnosis in patients with intrahepatic cancer confirmed by histology

Cancer diagnosis was obtained by fine-needle aspiration biopsy in 39 (78%) of the patients in whom the presence of cancer in the liver parenchyma was subsequently histologically proved (Table I). In one additional case suspected cancer cells were recorded. Thus a cancer diagnosis was *probably missed by the fine-needle aspiration biopsy* in 20% of the patients. Re-examination of these smears induced no revision of the cytodiagnosis.

Obviously in these false negative" cases, cancer cells had not been aspirated, although cancer was present at autopsy 2 weeks to 12 months later and had *probably* been present somewhere in the liver at the time of puncture.

There was no instance in which a fine-needle diagnosis of cancer could not be verified in the histological control thus no false positive. *In* two patients with liver cirrhosis conditions with proliferating liver epithelium however a *suspicion* of malignancy was mentioned in the report.

Cytodiagnosis in patients with a diagnosis of intrahepatic cancer based on clinical findings and course (autopsy not performed)

In the 24 patients in whom intrahepatic cancer in retrospect was regarded as highly probable, a cytodiagnosis of cancer was made in 18 (75%) (Table I).

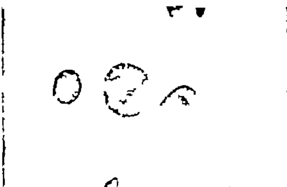
Cytodiagnosis in patients with a strong suspicion of intrahepatic cancer at the time of puncture

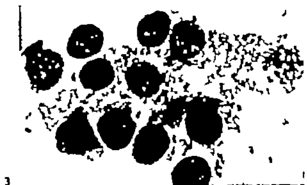
In 44 patients intrahepatic cancer was strongly *suspected because of the clinical picture* at the time of puncture. Of these, 35 (80%) showed cancer at the cytological examination. Cytodiagnosis was negative for cancer in six patients who later proved to have intrahepatic cancer. Despite the strong clinical suspicion of cancer three patients *finally proved to have liver cirrhosis, the presence* of which could be suggested from the cytological specimen.

Cytodiagnosis in patients without strong suspicion of intrahepatic cancer at the time of puncture

In numerous patients a diagnosis of intrahepatic cancer had to be considered as a point in the d f ferential diagnosis, especially when the material is studied in retrospect.

1 

 2

3 

 4

Fig 1 Metastatic cancer cells showing pronounced aniso-caryosis and pleomorphism. 800.

Fig 2 Binuclear giant cell from primary hepatoma. Note large nucleoli and blue-stained cytoplasmic inclusion. 900.

Fig 3 Metastatic tumour cells from carcinoid tumour in the small intestine. The purple-stained cytoplasmic *granules are very characteristic and permit the difficult* clinical diagnosis to be made merely on this cell group 800.

Fig 4 Two large malignant cells with multiple nucleoli and dark granules in the cytoplasm. The primary tumour *as malignant melanoma in the rectum.* 900.

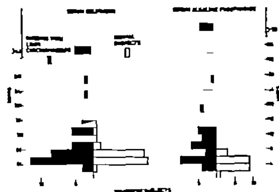


Fig. 5 Histogram demonstrating the serum concentration of total bilirubin and alkaline phosphatase in patients with hepatic cancer and in normal subjects.

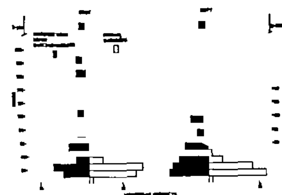


Fig. 6 Histogram demonstrating SGOT and SGPT in patients with hepatic cancer and in normal subjects.

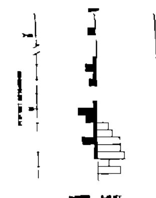


Fig. 7 Histogram demonstrating the bromsulphthalein retention in patients with hepatic cancer and in normal subjects 40–71 years of age.

Table I. *Cytodiagnostic results in 74 patients with malignant tumour in the liver. Figures within brackets represent incidence in per cent*

Cytodiagnosis	Pat. with cancer verified by histopathological examination	Pat. with cancer diagnosed through clinical follow-up	Total
Cancer	39 (78)	18 (75)	57 (77)
Suspected cancer	1 (2)	1 (4)	2 (3)
No cancer	10 (20)	5 (21)	15 (20)
Total	50	24	74

Table II. *Site of primary tumour in 74 patients with intrahepatic malignant tumour*

Primary site	No.
Lung	11
Liver	10
Stomach	10
Other sites	24
Unknown	19
Total	74

A cytodiagnosis of cancer was made in .2 patients in whom the clinical suspicion of malignant liver tumour was rather vague, and in many of these the diagnosis was a surprise. Two casuistic records may illustrate the clinical value of such findings.

Case 1 Female, 76 years. Presented with some weeks of tiredness. Five years earlier benign bronchial adenoma was extirpated, but since then the patient had been healthy.

On admission the physical examination was normal. The haemoglobin was 11.3 g per 100 ml, ESR 50 mm. The biochemical liver profile was within normal limits. Radiographic studies of the chest and of the upper and lower gastrointestinal tract did not reveal any abnormalities. The patient refused further investigations and went home.

She returned four months later complaining of subfebrile temperature and tiredness. Physical examination showed no change. The liver was *not palpable* and her weight was unaltered.

Laboratory data revealed: haemoglobin 10.9 g per 100 ml, ESR 100 mm, leucocytes 5 900 per mm³, differential count normal. Bilirubin, total 0.4 mg%. Protein 7.9 g% with strongly augmented alphaglobulin (1.57 g) and slightly elevated gammaglobulin (1.61 g). Alkaline phosphatase 14 units, SGOT 33 units, SGPT 36 units. BSP 4% retention. Serum B_{12} high, 7 300 *μμg* per ml. Urine normal. The stools did not contain blood.

Table III *Scintigraphic and cytodiagnostic results in 24 patients who 2–26 weeks later proved to have intrahepatic cancer*

Cancer diagnosis	Scintigraphy	Cytology
Positive	17	16
Suspected	4	1
Negative	3	7
Total	24	24

X-ray films of the chest, spine and pelvis revealed no change. A gastrointestinal series normal. Cholecystography intravenous pyelography and renal angiography normal.

After one month of rather advanced diagnostic work, an intercostal fine-needle aspiration biopsy revealed polymorphic cancer cells (Fig. 1) and necrosis. The tumour diagnosis was later confirmed by angiography of the coeliac artery and by scintigraphy

Case 2. Female 56 years Presented with two months weight loss and abdominal pains. At the examination an epigastric resistance was felt; this was questioned by several colleagues. There was no anaemia, ESR 23 mm. Total serum bilirubin 0.6 mg% serum alkaline phosphatase 12 units, SGOT 49 units, SGPT 18 units, BSP 17% retention. X-ray examination showed the digestive tract normal. Fine-needle aspiration biopsy from the left lobe revealed cancer cells. Peritoneoscopy offered good survey of rather small grey-brown liver with chagrinated surface, but without any tumour nodules; biopsy for histology obtained during this intervention resulted in the diagnosis of hepatitis with fibrosis. Another aspiration biopsy from the left liver lobe and cytological examination again showed cancer cells as before, and final diagnosis f multiple cancer metastasis in the liver was obtained in laparotomy five weeks after the first cytodiagnosis.

Cytodiagnosis in judging the origin of the tumour

In 55 patients it was possible to define *in retrospect* the origin of the tumour (Table II). It is well known that such a specification is difficult on cytological criteria alone, especially in view of metastatic tumours, in the present series metastatic tumours could be exactly defined only in two cases (carcinoid tumours and malignant melanoma) Instead, it was possible to make a specific diagnosis of *malignant hepatoma* in five of the ten cases represented (all males, 4 of whom had also a liver cirrhosis).

In the cases of malignant hepatoma, *general* cytological criteria left no doubts regarding the malignancy of the tumour Among features suggesting a hepatic origin were the rich occurrence of ( ) giant cells containing multiple nuclei or giant nuclei with polycyclic outline (*b*) cells with small satellite nuclei (c) extremely large nucleoli, staining deep blue (*d*) a specific type of larg., hyaline cytoplasmic inclusions, staining blue with May-Grünwald-Giemsa (Fig. 2) In the five cases reported here the cytoplasmic inclusions were absent in two, and the large nucleoli in one case; the three remaining cases presented the full tetrad of cytological signs mentioned here.

Small monomorphic nuclei surrounded by rather scanty ill-defined cytoplasm containing numerous purple-coloured granules (Fig. 3) were registered in one patient with metastatic *carcinoid tumour* In another patient fine black granules in the cytoplasm of the malignant cells (Fig. 4) permitted the diagnosis of metastatic *melanoma*.

Table IV *Arteriographic and cytodiagnostic results in 31 patients who 2–20 weeks later proved to have intrahepatic cancer*

Cancer diagnosis	Arteriography	Cytology
Positive	24	23
Suspected	0	1
Negative	7	7
Total	31	31

The Value of Fine-needle Biopsy Compared with Some Other Methods

Biochemistry

Most patients who subsequently proved to have intrahepatic cancer showed some degree of abnormity in the biochemical tests (Figs. 5 6 and 7). It should be noted, however that the test battery used was negative throughout in a considerable number of patients, and the observed deviations from normal were often of a very moderate degree. In agreement with Yesner and Conn (9) the author found that slight elevations of SGOT alkaline phosphatase, and BSP especially suggested the presence of malignant liver disease. It was noted that old age per se seemed to be associated with slightly elevated BSP which thus proved of little value as single observations.

Scintigraphy

Table III shows the scintigraphic and cytodiagnostic results in 24 patients in whom cancer was

later verified histologically Space occupying lesions were observed in 17 (71%), other abnormalities suspected of cancer in four The findings were negative in three.

Fine-needle biopsy had been performed independently of scintigraphic findings in all these cases (thus *not* directed towards foci detected by scintigraphy)· in 16 cases a cancer was found (67%)· in one case a cancer was suspected. The cases with positive cytology had also positive scintigrams.

Angiography

Selective hepatic arteriography was performed in 31 patients in whom cancer was later verified histologically Table IV shows that a correct diagnosis of tumour was obtained in 24 (77%) patients, in some, even the type of tumour could to some degree be specified.

Fine-needle biopsy was performed independently of radiographic findings in all these patients, and cancer cells were detected in 23 (74%), all identical with those diagnosed with angiography

DISCUSSION

It is concluded from the observations in this series without any accidents that fine-needle biopsy can be obtained *without complications* even in the presence of malignant tumour in the liver Thus the hazards reported with conventional biopsy technique for histology (4, 5 7) do not to any extent *invalidate the fine-needle technique*. This is in accordance with the author's experience in an extensive material of liver punctures performed on other indications. The fine needle has an advantage over the conventional coarse needles because it can be used for probing suspected spots anywhere in the liver e.g. in the left lobe, where the use of coarse needles might be risky

Furthermore, it is concluded that cytodiagnosis by fine-needle biopsy is a *reliable* method of diagnosing intrahepatic malignant tumour There was no false positive in this series, and the number of patients with intrahepatic cancer not detected by this method, was low

The present study was made in retrospect, and *unfortunately angiography and scintigraphy* were done in only limited number of patients. The diagnostic yields of fine-needle puncture, angiography and scintigraphy proved to be practically the same; the cases positive with angiography and scintigraphy were identical with those independently detected also by cytodiagnosis. The ex*planation must be that all three methods detect* mainly rather advanced degrees of intrahepatic tumour growth, i.e. large tumour masses or wide spread tumour infiltration.

The observations in this study are in accord with the old experience that even advanced degrees of intrahepatic tumour growth may be clinically silent. This is evident also from the biochemical tests presented in this paper

What then is the place of fine-needle biopsy in a clinical diagnostic schedule for the detection of malignant tumour in the liver

It was seen above that it detects about the same percentage of tumours as angiography and scintigraphy but fine-needle biopsy has two intrinsic advantages over these methods that must be taken into account.

(*a*) Its conclusions are based on the findings of malignant tumour cells a positive finding is thus a final argument.

(*b*) It is a technically very simple "bedside method, always available without delay even in the absence of special laboratory facilities.

There are three clinical situations where fine needle biopsy is especially helpful.

1 Where the case history and the clinical picture are *strongly suggestive of intrahepatic tumour growth*. In these situations fine-needle puncture is *the easiest way to confirm the diagnosis with* high yield of positive findings. In the present investigation it was found to have an 85% chance of confirming the diagnosis. It can be used as first method to be followed by scintigraphy or angiography on special indications.

2 Where the case history and the clinical picture *do not suggest* intrahepatic cancer but this possibility among others, should be considered in general examination programme. In such cases fine-needle biopsy can be included in the diagnostic schedule as simple screening procedure.

3 Where foci detected, e g. by scintigraphy and angiography need further definition with regard to their nature. This can be achieved by direct fine-needle puncture of such foci for cytological diagnosis.

As in punch biopsy for histology a pecific

classification of the tumour in the smear with regard to its origin is possible in relatively few cases. However it is interesting to note that, in five out of ten patients, fine-needle biopsies suggested primary hepatoma, which was later verified by autopsy Several morphological details contributed to this diagnosis. As typical appearance of hepatomas, however I would stress the hyaline cytoplasmic bodies which seem to be specific for hepatoma cells and to the best of my knowledge have not previously been described. Cytomorphological differentiation of hepatoma could be of special importance because today surgical treat ment is possibly within reach in some cases.

The appearance of carcinoid tumour cells was very characteristic. In a later material this diagnosis has been made cytologically on two further occasions.

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LIVER BIOPSY WITH A NEEDLE OF 0.7 MM OUTER DIAMETER

Safety and Quantitative Yield

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Abstract. The study presents investigation potentialities and risks of liver biopsy method with very fine needle (0.7 mm outer diameter). In 2 611 biopsies a serious complication was seen in one case, an intrahepatic haematoma, which had to be evacuated surgically. Otherwise even minor inconveniences to the patients proved exceptional. Specimens for cytological examination were obtained in 99% of the biopsies, and in 34 of 41 biopsies fragments of coherent liver tissue were obtained with protein content varying from 32 to 900 µg. The specimens proved satisfactory for electron microscopy and under certain conditions also for chemical analysis of liver tissue.

In the clinical diagnosis of liver diseases some kind of microscopic or biochemical analysis of liver tissue is often necessary. Liver biopsy specimens have usually been obtained by surgical excision at the time of abdominal operation or percutaneously with various needles relatively large in diameter, in this study called punch biopsies. These procedures for liver biopsy are not without risk (7); this has restricted their use, particularly in investigative studies.

As regards the "blind" puncture methods, there is reason to assume that hazards due to the puncture trauma (haemorrhage, bile leakage, peritonitis) are to a certain extent proportional to the caliber of the instrument used. The author has used needles with an outer diameter of only 0.7 mm with the aim to reduce the incidence of complications as far as possible. This is a prerequisite for the use of liver biopsy in *serial* investigations of short-term reactions of liver parenchyma in different metabolic situations.

This paper presents some details regarding the biopsy technique, the hazards, including minor discomfort to the patients, and the quantity of liver tissue obtained.

MATERIAL AND METHODS

Complications were evaluated on observations during 24 hours after puncture in 2 611 punctures performed by the author in 2 305 patients during 1964–1969.

Subjective discomfort was evaluated in 93 consecutive subjects.

Quantity of liver tissue obtained was studied in 41 biopsies from 23 healthy subjects.

Instrument. The biopsy was performed with a 10 ml syringe (Fig. 1) with a handle permitting single-hand operation (AB Kifa, Stockholm). Ordinary well-fitting 22-gauge needles (Söderberg & Co., Göteborg), 12 cm long with sharp-ground edge without obturator were used.

Care of the patient. There was no premedication. Local anaesthesia was used only when immediate repetition of the puncture was planned or for psychological reasons. Haemorrhagic diathesis was considered a contraindication to biopsy; the platelet count was routinely controlled and sometimes also bleeding time and clotting time. Bed rest for one hour was advised after biopsy.

The *biopsy technique* has been described by Söderström (9); here only some details and modifications will be stressed. Biopsy was usually performed intercostally between the ventral and the mid-axillary line at the site of maximum dullness to percussion. With the patient holding his breath in expiration, the needle was rapidly introduced into the liver during simultaneous strong aspiration, which had to be stopped before the point of the needle had left the liver. The shorter the time the needle remained in the liver, the less the blood admixture to the specimen. For *cytodiagnosis* the aspirate was immediately distributed in thin smears on several slides and allowed to dry. For *electron microscopy* the specimen was fixed in cold 2% buffered osmium tetroxide (pH 7.3) and embedded in epoxy resin (Epon 812).

When *biochemical analysis* was intended, the biopsy was expressed in a glass vessel and quickly washed free from blood in a suitable liquid. Fragments of solid tissue present were collected with a needle and transferred for further processing as previously described (4).

The problems encountered in gravimetric estimates of small tissue specimens, particularly due to the presence of washing fluid and evaporation, were avoided by cal-

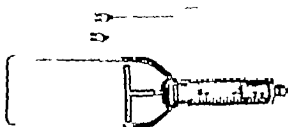


Fig 1 Needles of various lengths and the syringe designed by Franzén.

calculating the weight from estimates of protein, presuming the protein content to be 20% of the wet weight (5).

Protein was measured according to Lowry et al. (3).

RESULTS

Complications

In the entire series of 2 611 biopsies, mostly performed for cytodiagnosis, there was no mortality but one serious complication

A 61 year-old woman denying bleeding tendency was biopsied intercostally without immediate incident. Six hours later she felt abdominal distension and gradually increasing slight epigastric pains. Three days later she presented with an enlarged tender liver hypotension and anaemia. At the puncture site an intracutaneous haemorrhage measuring 20 mm in diameter was noted. Platelet count was 38 000 compared with 230 000 before biopsy The thrombotest value was 9% rising to 80% after blood transfusion. Three days later an intrahepatic haematoma had to be surgically evacuated. After recovering, she refused further analysis of the haemostatic mechanism.

This case is exceptional in this series the author cannot explain the incident on the basis of available data, but some sort of thrombocytopathy or coagulation defect cannot be excluded. Otherwise the untoward reactions registered were restricted to abdominal pains lasting for one or two days in 14 patients, nine of whom suffered from biliary obstruction. (In the total material 55 patients had an overt bile stasis.)

There was no instance of pneumothorax, pleuritis or peritonitis. In 15 patients without bile stasis, bile admixture, presumably from larger bile ducts, necessitated repetition of the puncture in another intercostal space, but gave no trouble to the patients. In 12 patients the specimens contained material from the colon or the kidney usually in addition to liver cells. No perceptible reactions were noted after these accidents, which might have been of serious consequence using a conventional high-gauge biopsy needle.

It is a professional habit of doctors to forget about minor untoward reactions to interventions advocated by themselves, which later prove to be transient. The patients are more trustworthy censors on this point and for this reason patients were interviewed for the occurrence of pains or other discomfort four hours after 93 consecutive biopsies. The biopsies in this series included two punctures for material to be treated in different ways. Slight ache in the right side of the abdomen or the back was reported by three patients for up to three hours a transient nausea one hour after the puncture was noted by one patient. Fifty-nine probands classified the puncture pain as equivalent to or less than that of a vein puncture 31 as somewhat more painful. Questioned whether they would prefer "the next liver test" to be performed by puncture of a cubital vein or by liver puncture, 25 preferred vein puncture, 56 had no definite preference, and 12 preferred the abdominal puncture, although the vein puncture had previously been performed by a well-trained assistant.

Quantity of tissue material

The minimum amount of liver cells deemed to be necessary for a cytodiagnosis in smears was obtained in 2 580 biopsies (99% of the total series). In 41 consecutive biopsies the quantity of coherent parenchymal tissue (Fig 2) to be obtained by the method was studied by determination of the pro-



Fig Liver biopsy specimen (1.0 25 mm) obtained with Menghini needle of 1.4 mm diameter compared with the specimen obtained with 0.7 mm needle.

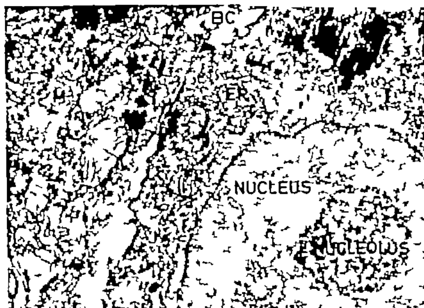


Fig. 3. Electron micrograph of liver specimen showing rough endoplasmic reticulum (ER), numerous mitochondriae (M) and bile canaliculus (BC). The fine dark stippling of the liver cell cytoplasm is due to glycogen particles. From patient intoxicated with methylene dichloride (21, 750).

tein content of such fragments. This varied in 34 biopsies between 32 and 900 µg, mean 225 µg, corresponding to a wet weight of 0.16–4.5 mg, mean 1.12 mg. In seven specimens material sufficient for such determinations was not obtained. I have noted that rich and satisfactory material was more often obtained from elderly well-nourished subjects than from young, slender individuals. I cannot explain this difference, which is, however, of some practical consequence. Fig. 2 demonstrates the fragments of solid tissue obtained with a needle of 0.7 mm outer diameter compared with punch-needle specimen (1 × .5 mm) obtained with a Menghini needle of 1.4 mm diameter.

Electron microscopy

Electron microscopy has so far been performed on ten biopsy specimens. The results demonstrate that the method can offer satisfactory specimens for electron microscopy (Fig. 3). (Dr Claes von Mecklenburg, Dept. of Zoology, University of Lund, performed the electron microscopical work.)

DISCUSSION

This study was planned as a presentation of fine-needle liver biopsy as an absolutely risk-free procedure when the single case of intrahepatic haematoma reported made me conclude that an absence of hazards can never be taken for granted with any method of blind liver biopsy. However, my experience proves that a fine needle really does expose patients to a minimum of risks, even compared with the surprisingly favourable figures published by Lindner (2) in a combined series of punch-needle biopsies performed mostly by experienced operators. In material of 79 381 biopsies the mortality was 0.015 % and serious complications occurred in 0.34 %, thus nevertheless about ten times the complication rate observed in the present study. The complication rate has, however, been considerably higher in other series published, especially the smaller ones (6, 8, 12). The risk in needle biopsy methods is probably inversely proportional to the experience of the operator; with the fine needle even a wrong target hit by an inexperienced operator will be a mishap without serious consequence.

Hazards inherent in clinical methods should be justified by the value of the information obtained. Can fine-needle liver biopsy from this viewpoint defend place in diagnostic or investigational work?

Fine-needle liver biopsy was originally used for *cytodiagnosis* of liver disease; its performance in this field may well justify its use of wide indications. In this study I have emphasized the fact that the fragments of solid liver tissue usually

obtained may well be used also for other purposes than cytodiagnosis in smears. The amount of solid tissue present in a *single aspirate* varied on average the weight was about 1/10 of the material usually obtained with a Menghini needle of 1.4 mm diameter (10) According to earlier approximate estimates (1) this implies a cell content of 30 000–900 000 in the 34 biopsies studied from this viewpoint in the present paper

This quantity is sufficient for many types of biochemical analysis of liver tissue. The present material has thus been used for determination of various enzymes (4 5) In another paper time sequence studis of the accumulation of triglycerides in human liver after ethanol ingestion have been reported (11).

It is evident from my figures that solid specimens were not obtained in about 1/5 of the aspirate. The remedy against this drawback is a new puncture in the same session, if necessary a third one with the fine-needle technique such *repeated punctures are certainly justified. There*fore specimens for cytological evaluation by light microscopy or electron microscopy can in practice always be secured. Using the coarse-needle technique the justification for repeated punctures after dry taps is debatable (7) If the reason for the failure is interposition of other tissue (colon, kidney) the consequences of repeated punctures may be serious. The safety of fine-needle biopsy makes it a method of choice when repeated punctures at short intervals are needed, by means of which the course of metabolic processes can be followed in biochemical and/or morphological studies.

The problem of finding a standard of reference in quantitative analyses suitable to normal as well as pathological livers has not been solved. In this investigation protein was chosen as reference base, but other substances such as phospholipids or DNA might be more advisable in special situations.

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VERTEBRAL ARTERY INSUFFICIENCY AND ROTATIONAL OBSTRUCTION

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Abstract. A case of intermittent vertebro-basilar insufficiency is reported. The combination of a hypoplastic right vertebral artery and marked rotational obstruction of the left one, which had an anomalous origin, seems to have been the cause of the compromised blood flow. Surgical treatment of the presumed contributing obstructive factor was tried. The value of performing arteriograms in various positions of the head is emphasized.

During the last decade various causes of intermittent vertebrobasilar insufficiency have been reported. Intraluminal atherosclerosis contributes to compromised blood flow. Other causes, such as subclavian steal (11), and various extraluminal factors (2, 10, 12), have been found to impair vertebral circulation.

In some patients attacks of intermittent cerebral ischemia may be precipitated by sudden movements of the head and neck. Turning the head to the side or upwards are the most commonly precipitating movements. The attacks are transient and consist of such symptoms as dizziness, vertigo, gait disturbances, nausea, fainting, headache, visual disturbances, and numbness or tingling of the upper limbs.

Among patients with this syndrome we have observed one in whom the vertebral blood flow was impaired in a way not reported before. We find the syndrome of clinical significance and to justify this report as a reminder of the problem.

CASE REPORT

The patient was a man born in 1928, working as a clerk. He had been well until 1964, when he had a period of attacks of intense occipital headache. In 1965, while gardening, he suddenly had an attack of vertigo and vomiting. The symptoms disappeared slowly. At examination one week later horizontal nystagmus and abnormal finger-nose test were found. Examination some months later revealed horizontal nystagmus and vertigo when his head was turned to the right.

After that the patient had frequent attacks of dizziness, vertigo, nausea, and occipital headache. Sometimes he was so disabled by these symptoms that he resorted to complete bed rest for some days. No symptoms of sensory changes or of intermittent claudication were experienced from the limbs. The symptoms appeared in connection with movements of the head, especially turning the head to the right and upward. Sometimes he had symptoms when driving his car. Especially looking to the right and backward when backing the car gave him attacks of vertigo.

In September 1966 the patient was admitted to our hospital because of a new attack. He complained of intensive vertigo and of occipital headache. There was horizontal nystagmus to the left. The signs and symptoms were much increased when his head was turned to the right. The physical examination revealed no other abnormal findings. Auscultation of the heart revealed nothing abnormal. No supraclavicular bruit was heard. The blood pressure was normal and equal in both arms. The hematological status was normal. Total serum cholesterol was 220 mg per 100 ml. Urine analysis was normal. Nothing abnormal was found at X-ray of the skull and cervical spine.

Arteriographic findings

Aortography was performed with the catheter introduced through the right femoral artery to the aortic arch. During injection of 60% Urografin® the aortic arch and the great arteries of the head and neck were visualized. The right vertebral artery was found to be quite hypoplastic. The left vertebral artery was of normal width but had an anomalous origin (Fig. 1). It was found to have its origin directly from the aortic arch medial to the origin of the subclavian artery. No other abnormalities of the aortic arch or of the other great arteries were found.

When arteriograms were obtained with the head turned to the right and upward, the filling of the left vertebral artery was much impaired, especially in its upper part. During this manoeuvre the patient almost lost consciousness. He also suffered nausea and transient nystagmus.

Treatment

As the left vertebral artery was found to be impaired upon forced rotation of the head, surgical exploration of the left side was performed. The operation was made in order to free the artery from tendinous interdigitations, which



Fig. 1. Arteriogram, anteroposterior projection, showing the hypoplastic right vertebral artery and the left artery of anomalous origin.

were supposed to effect mechanical occlusion of the artery prior to its entry into the vertebral canal (5). A supraclavicular approach was used. The scalenus anticus muscle was found partially to obstruct the subclavian artery. The muscle was divided. The vertebral artery was mobilized up to its entering the vertebral canal. The artery was found to be rather wide. No obliteration by fascial bands or by interdigitations between the scalenus anticus or longus colli tendons could be detected. On turning the patient's head to the right, however, the left vertebral artery was found to be markedly bent over the rather prominent longus colli muscle. This bending and stretching of the artery was found to affect the blood flow. On that account, part of the otherwise normal longus colli muscle was resected, giving free passage to the artery.

During three years after the operation the patient has had occasional attacks of headache, but no more attacks of vertigo. Evaluation of the result may be uncertain because of the spontaneous periods of remission in cases like this. He has at all events been able to do his usual work all the time and has felt much improved. Symptoms do not appear any more when he is driving his car. No signs or symptoms can now be elicited by forced rotation of the patient's head. The patient has denied aortography postoperatively.

COMMENTS

Changes of the blood flow through the carotid and vertebral arteries normally arise on movements of the head (13). Particularly lateral rotation and extension of the head have been found to reduce the blood flow through the contralateral arteries (3). Rotational obstruction may be seriously aggravated by intraluminal atherosclerosis and by extraluminal factors such as fascial bands (4), osteophytes (6), anomalous origin of the vertebral artery (9), and contraction of adjacent muscles (5). Normally the collateral pathways through the arteries of the other side and through the circle of Willis ensure adequate supply of the brain when *rotational* changes occur (7). The compensatory mechanisms may however be impaired by grossly diseased or congenitally defective arteries. In our case the combination of the hypoplastic vertebral artery at one side and the rotational obstruction of the other which had an anomalous origin seem to have caused changes of the blood flow causing intermittent symptoms of brain stem ischemia.

As has been pointed out by Powers et al. (8) the intermittent symptoms of vertebral artery insufficiency consist of four principal complexes: (*a*) cochlear-vestibular symptoms including vertigo, tinnitus, and nerve deafness; (*b*) vascular headache, usually unilateral and pounding in character with or without an aura, and frequently associated with blackout spells; (*c*) visual symptoms of the perceptive type, including diplopia, visual claudication, and transient bilateral field defects; and (*d*) numbness and paresthesias of the arms.

Intraluminal atherosclerosis may be treated by endarterectomy or bypass (1). Periarterial sympathectomy too, has been emphasized as treatment of vertebral insufficiency (8). Concerning rotational obstruction of the vertebral artery surgical treatment of the various factors contributing to the impairment of the blood flow has been reported to be of value (4, 5, 9, 14). To establish such factors, a detailed case history and minute examination including posture test must be supplemented by arteriography of the great arteries of the head and neck. The arteriograms ought to be taken not only in the anteroposterior projection, but also in the "head drop" and in the rotational positions of the head.

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A CLINICAL DIAGNOSTIC INDEX IN THE DIAGNOSIS OF THE DUMPING SYNDROME

Changes in Plasma Volume and Blood Sugar after a Test Meal

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Abstract. On the basis of the literature and personal analysis of symptoms and signs in 25 gastrectomized patients, new criteria for the dumping syndrome have been outlined. The clinical diagnosis of the dumping syndrome was based upon clinical diagnostic index. The method was then applied to 49 partially gastrectomized patients. It is stressed that three of the cardinal symptoms may easily be overlooked, viz. sleepiness, dizziness, and restlessness. Eructation and vomiting are components of the afferent loop syndrome and the small stomach syndrome. After test meal of hypertonic glucose the percentage changes in plasma volume were measured by Evans blue, hematocrit, and hemoglobin values. All three methods revealed statistically significant differences between dumpers and non-dumpers as regards the mean values of the percentage fall in plasma volume. An increasing fall in plasma volume was generally paralleled by rising clinical diagnostic index. All patients who showed fall in plasma volume above 15% were dumpers. Women showed greater fall in plasma volume and distinctly larger clinical diagnostic index than men. Statistically significant differences were found between dumpers and non-dumpers as regards the rise in blood sugar after the test meal.

In the past almost all postgastrectomy symptoms were usually included in the dumping syndrome without regard to etiology. During the last years both clinical observations and animal experiments have contributed to a better understanding of the pathophysiology of the dumping syndrome.

Many writers have regarded eructation and vomiting as components of the dumping syndrome, but these symptoms are components of the small stomach syndrome or the afferent loop syndrome. A typical feature in most dumpers is their inability to vomit. Many patients suffering from pure afferent loop syndrome or small stomach syndrome are troubled by nausea and distension in the upper part of the abdomen. Their general condition may be affected, they complain of weakness and look pale and clammy during vomiting. Thus some of their symptoms are identical with the symptoms of dumping.

Some important dumping symptoms may easily be overlooked, viz. sleepiness, drowsiness, apathy dizziness, and restlessness. During a period of drowsiness or apathy the patient may be unable to observe and describe dumping complaints.

Some authors demonstrated a correlation between the amount of decrease in plasma volume and the dumping symptoms (1 11 16, 20, 24 29 30 34 39). Other workers, however found the plasma volume depletion inadequate to explain all the dumping symptoms (14 19 28) and finally a third group of authors found no correlation between the fall in plasma volume and the dumping symptoms (4, 7 13 40).

While some workers found no relation between the blood sugar level and the dumping symptoms (1 13 25 3[illegible], 33 41), others showed a correlation between the blood sugar rise and the dumping symptoms (3 10, 17 21 [illegible], 37) and some of them demonstrated a correlation between the decrease in plasma volume and the increase in blood sugar (2, 10, [illegible]1). Precibal administration of hypoglycemic agents reduced the postcibal hyperglycemia, with concomitant decrease in the fall of plasm olume, at the same time relieving the symptoms (3 [illegible]) Patients with the dumping syndrome ha been treated with hypoglycemic agents with promising results (2, 3 2[illegible], 36, 38). A

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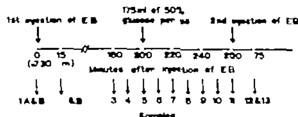


Fig. 1. The points of time for injection of Evans blue, blood sampling, and test meal. EB, Evans blue; A & B, two blood samples drawn in succession.

defect of carbohydrate metabolism may be a factor in the production of the dumping syndrome (3, 11).

The purpose of the present investigation was to outline new criteria for the dumping syndrome and to study whether there is any correlation between the decrease in plasma volume and the production or severity of the dumping syndrome. A comparison is also made between dumpers and non-dumpers as regards the rise in blood sugar after the test meal.

MATERIAL

The material comprised 49 patients who had been partially gastrectomized because of peptic ulcer: 26 dumpers (18 men and 8 women) and 23 non-dumpers (15 men and 8 women).

The clinical diagnosis of dumping was based upon a clinical diagnostic index as shown below. No significant difference was found between dumpers and non-dumpers as regards age, length of follow-up interval after gastrectomy, height, weight at operation, loss of weight after operation, fasting plasma volume and distribution of gastric and duodenal ulcer.

Table I. *Weighting factors allocated to the symptoms and signs of the dumping syndrome*

A clinical diagnostic index of +7 or above indicated dumping, indices of −4 or below non-dumping.

Pre-shock, shock	+5
Almost fainting, syncope, unconsciousness	+4
Desire to lie or sit down	+4
Breathlessness, dyspnea	+3
Weakness, exhaustion	+3
Sleepiness, drowsiness, yawning, apathy, falling asleep	+3
Palpitation	3
Restlessness	+2
Dizziness	+2
Headache	+1
Feeling of warmth, sweating, pallor, clammy skin	+1
Nausea	+1
Fullness in the abdomen, meteorismus	+1
Borborygmia	−1
Eructation	−1
Vomiting	−4

METHODS

Clinical diagnostic index

In order to keep the personal judgment at a minimum, the clinical diagnosis of the dumping syndrome was based upon a clinical diagnostic index. This index was established in the same way as the clinical diagnostic index for hyperthyroidism, described in 1959 by Crooks et al. (12). The criteria were given a quantitative character.

All patients were examined in hospital. They were asked specifically about the diagnostic symptoms of the dumping syndrome which occurred after meals at home. As leading questions always introduce the risk of suggestion, the patients were cross-questioned in various ways, and some "blind" questions were also inserted.

The test meal. The patients drank within one minute 175 ml of 50% glucose flavored with lemon (Fig. 1). The same amount of glucose was used independently of the weight of the subject. The test meal was given in the forenoon, after the patient had been without food or drink for 16 hours. During the test meal the patient was semi-recumbent on a bed-rest tilted to 45 degrees.

The patients were observed by the author continuously during the first hour after the test meal. The pulse rate was counted before the test meal and at intervals of 10 min during the first hour after the test meal. The number of beats was counted for 1 min in one of the radial arteries. In every patient I used the pulse index, which expresses the pulse as a percentage of the pulse rate counted immediately before the meal. The blood pressure was measured when a patient seemed to be in a state of pre-shock or vaso-vagal attack.

In a pilot study comprising 25 partially gastrectomized patients (20 men and 5 women) the clinical features were weighted by allocating a score to each. The positive or negative values of these scores were initially allocated on the basis of the relative diagnostic significance of each symptom and sign as this appeared in the literature. The clinical diagnostic indices, or total scores, were then calculated. These scores separated dumpers and non-dumpers, but the weighting factors for the individual clinical features were further modified to produce the widest possible separation between the two groups. Cardiovascular symptoms and symptoms characterized as cerebral dumping were given high positive weighting factors, intestinal symptoms low factors (Table I). The intestinal symptoms alone do not constitute the dumping syndrome (15). Vomiting was given a high negative factor. The indices were then re-calculated.

The clinical diagnostic indices of 18 unquestionably dumping subjects were greater than +6. Seven unequivocally non-dumping subjects had clinical diagnostic indices of less than +5.

The method was then applied to 49 patients who had been partially gastrectomized because of peptic ulcer (33 men and 16 women). Only three patients had clinical diagnostic indices in the equivocal range +5 to +6. On account of the higher mean value of the clinical diagnostic index in women, the two women in this doubtful group were diagnosed as non-dumpers. The third subject in the doubtful group was a man who was diagnosed as dumper. Thus the material comprised 18 male and 8 female dumpers, and 15 male and 8 female non-dumpers.

Table II. *The incidence of the current symptoms and the test symptoms*

	Number of patients							
	Dumpers				Non-dumpers			
	Males		Females		Males		Females	
Symptoms	Current sympt.	Test sympt.	Current sympt.	Test sympt.	Current sympt.	Test sympt.	Current sympt.	Test sympt.
Pre-shock, shock	0	1	0	5	0	0	0	0
Almost fainting" syncope, unconsciousness	2	0	4	3	2	0	3	0
Desire to lie or sit down	12	1	7	2	6	0	6	0
Breathlessness, dyspnea	3	3	3	7	3	1	1	0
Weakness, exhaustion	13	15	8	8	7	0	7	2
Sleepiness, downstate, apathy falling asleep	6	12	3	6	1	2	1	1
Palpitation	10	8	7	5	6	3	5	1
Restlessness	0	5	0	1	0	0	0	0
Dizziness	4	4	4	1	4	1	1	1
Headache	2	5	2	5	0	0	0	2
Feeling of warmth, sweating, pallor, clammy skin	9	5	7	6	5	3	3	0
Nausea	9	12	4	7	6	3	3	5
Fullness in the abdomen, meteorismus	6	7	2	1	1	3	2	0
Borborygmia	4	4	4	3	2	1	2	1
Eructation	1	5	0	3	2	3	2	5
Vomiting	3	0	1	3	5	1	3	0

Changes in plasma volume

Both in the morning and one hour after the test meal the plasma volume was determined by the direct method of Evans blue (35) (Fig. 1). The percentage changes in plasma volume after the test meal were measured by the indirect method of Evans blue (35). The percentage changes in plasma volume were also calculated by means of haematocrit and hemoglobin values (35).

Blood sugar index

The blood sugar analyses were carried out in venous blood samples (Fig. 1). Determination of blood sugar concentration was done by the method of Hagedorn et al. (18). The increase in blood sugar after the test meal was characterized by the blood sugar index, which expresses the blood sugar concentration in a sample as percentage of the concentration in the sample just before the test meal (21, 37).

RESULTS

Comparison of the symptoms after the test meal with the symptoms as determined from the patients' histories

A comparison was made between the incidence of different test symptoms and symptoms ascertained through the questionnaire (Table II).

Pre-shock or shock was observed in six patients (5 women and 1 man) during the test. The patients showed a marked increase in pulse rate and a decrease in blood pressure. This is a specific clinical sign observed by a physician. Some of the patients at home avoided carbohydrate-rich food and lay down after heavy meals. One of the women, who looked like a patient in shock, had an initial increase in pulse rate which was followed by a decrease in pulse rate and blood pressure. The reaction was regarded as a vaso-vagal attack.

In the different groups of patients I calculated

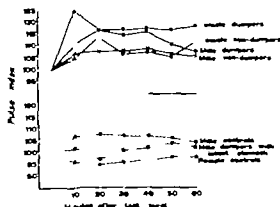


Fig. 2. Comparison between the mean values of the pulse indices in different groups of subjects.

Table III *Comparison between the clinical diagnostic indices after ordinary meals and after the test meal*

	Clinical diagnostic index	
	After ordinary meals	After the test meal
Male dumpers		
Mean	9.8	9.4
Range	0–20	6–18
Female dumpers		
Mean	16.3	16.8
Range	10–22	10–27
Male non-dumpers		
Mean	5.5	1.0
Range	−3– 19	0–4
Female non-dumpers		
Mean	9.5	2.1
Range	−3–+24	0–6

the means of the pulse indices which corresponded in time (Fig. 2). A statistically significant difference was observed between female dumpers and non-dumpers as regards the pulse indices 10 min after the test meal $(0.005 > P > 0.001)$ and the maximal individual pulse indices $(0.02 > P > 0.01)$

Weakness and exhaustion were the most frequent symptoms in the dumping syndrome. All female dumpers and most of the male dumpers experienced weakness after ordinary meals and the test meal. The patients complained of general muscular weakness or weakness all over the body

Sleepiness, drowsiness, yawning, apathy falling asleep and restlessness were observed more frequently after the test meals than after ordinary meals. All these features are easily noticed by the physician, but difficult to observe by the patients themselves.

After the test meal many patients got drowsy absent-minded, yawned all the time and had difficulties in replying to questions. Some were restless, continually moving their arms and feet and changing position in bed. Some of them fell asleep. They often said that they had been perfectly well, even though the investigator had observed a definite reaction. If the investigator had not been present all the time, the reaction might have been overlooked and the test characterized as negative.

Many patients desired to lie or sit down after ordinary meals. This was obviously a rare symptom after the test meal when the patient was lying in bed in semi-recumbent position.

Billous vomiting was noticed in four dumpers after ordinary meals and in three after the test meal. Billous vomiting suggests the presence of the afferent loop syndrome. The dumping syndrome and afferent loop syndrome sometimes, however occur together and the two syndromes may vary in intensity (6).

Both in dumpers and non-dumpers women showed a distinctly higher mean clinical diagnostic index than men (Table III) This is in agreement with other authors (8, 20, 26) who found that women are more severe dumpers than men. In dumpers the mean clinical diagnostic index was of the same magnitude after ordinary meals and test meals. In non-dumpers the mean clinical diagnostic index was higher in the case history

One-third of the current dumpers (11 of 33) were symptom-free during the test. One-fourth of the symptom-free gastrectomized persons (4 of 16) experienced dumping symptoms during the test.

Changes in plasma volume

The mean percentage changes in plasma volume have been recorded in Figs. 3 and 4 Good agree-

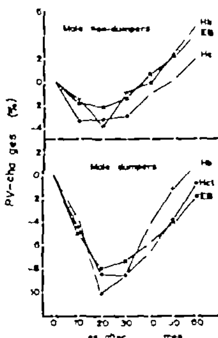


Fig. 3 The mean values of the percentage changes in plasma volume. The calculations performed in 15 male non-dumpers and 18 male dumpers by the indirect method of E ans blue (EB), hematocrit (Hct), and hemoglobin (Hb) values.

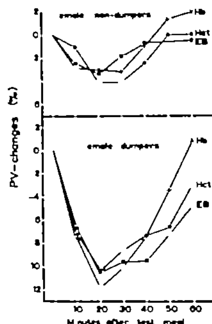


Fig. 4. The mean values of the percentage changes in plasma volume. Eight female non-dumpers and eight female dumpers.

ment was observed between the mean values obtained by the three methods. The simple hematocrit and hemoglobin methods seemed to be well fitted.

The fall in plasma volume was distinctly greater in dumpers than in non-dumpers. All three methods revealed the following statistically significant differences between the mean values of the percentage fall in plasma volume:

A highly significant difference ($P < 0.001$) was revealed between the maximal individual fall in plasma volume in male dumpers and non-dumpers. Twenty and thirty min after the test meal there was significant difference ($P < 0.05$) between the two groups.

A significant difference was disclosed between female dumpers and non-dumpers as regards the maximal individual fall in plasma volume and the fall in plasma volume twenty min after test meal.

At the end of the test the direct method of Evans blue showed highly significant difference between male dumpers and non-dumpers.

The 49 patients were divided into three groups on the basis of the maximal fall in plasma volume (Table IV). Patients with a small decrease in plasma volume (below 7%) had moderate or no symptoms. Most of them were non-dumpers.

When the fall in plasma volume was moderate

Table IV. *The clinical diagnostic indices in 33 gastrectomized men and 16 gastrectomized women, divided into three groups, according to the maximal individual fall in plasma volume*

EB = Evans blue. Hct = hematocrit. Hb = hemoglobin

Maximal plasma volume fall determined by	No. of subjects	Clinical diagnostic index: Mean	Clinical diagnostic index: Range
Men			
Below 7%			
EB	18	3.3	0– 8
Hct	14	3.4	0–18
Hb	13	2.1	0– 8
7–15%			
EB	12	7.6	0–15
Hct	15	6.1	0–13
Hb	16	6.9	0–18
Above 15%			
EB	3	11.0	7–18
Hct	4	11.5	9–15
Hb	4	11.8	9–15
Women			
Below 7%			
EB	7	2.4	−1–+6
Hct	6	1.8	−1 –5
Hb	5	1.2	−1– 4
7–15%			
EB	7	13.7	0–23
Hct	7	13.1	0–23
Hb	7	11.7	0–23
Above 15%			
EB	2	19.0	11–27
Hct	3	16.0	10–27
Hb	4	16.0	10–27

(7–15%), the mean clinical diagnostic index was distinctly higher but there was a wide range. A few patients in this group were symptom-free while others had severe dumping symptoms.

In a small number of patients the plasma volume decreased more than 15%. All of them were dumpers, and the mean clinical diagnostic index was higher than in the former group.

Thus, when a comparison was made between the three groups of patients, an increasing fall in plasma volume was parallelled by a rising clinical diagnostic index.

Figs. 5 and 6 show the correlation between the maximal fall in plasma volume and the clinical diagnostic index. In many patients there was a good correlation. There was, however no complete correlation, and in some patients there was discrepancy.

Women showed a greater fall in plasma volume

Fig. 5 Graph showing the relationship between the maximal percentage fall in plasma volume (mean value of the three methods) and the clinical diagnostic index in 15 male non-dumpers and 18 male dumpers.

than men. This difference was observed both in dumpers and non-dumpers, and by all three methods. Women showed a distinctly larger clinical diagnostic index than men, both in the case histories and during the test meals (Table III), yet the maximal fall in plasma volume was only slightly greater in women. This may be due to the fact that women have a smaller plasma volume than men.

The fall in plasma volume generally coincided time with the patients symptoms. In female mpers the symptoms started earlier than in male s. The symptoms started on an average 4.9 min (1–10) and 9 1 min (3–25) after the test meal in the two groups, respectively The maximum symptoms occurred on an average 22.0 (11–50) and 22.3 (9–40) min after the test meal in

Fig 6. Graph showing the relationship between the maximal percentage fall in plasma volume (mean value of the three methods) and the clinical diagnostic index in 8 female non-dumpers and 8 female dumpers.

female and male dumpers, respectively This is in good agreement with the maximal fall in plasma volume which was observed 20–30 min after the test meal by the different methods (Figs. 3 and 4).

Changes in blood sugar

A comparison was made between the mean values of the blood sugar indices which corresponded in time (Fig. 7) Dumpers showed a more rapid rise and higher maximal values of the blood sugar index than non-dumpers.

There was a statistically significant difference ($P < 0.05$) between female dumpers and non-dumpers as regards the maximal individual blood sugar index and the blood sugar indices 10, 40, 50 and 60 min after the test meal.

The difference was statistically significant between male dumpers and non-dumpers as regards the blood sugar indices 10 30 and 40 min after the test meal.

There were, however considerable individual variations with marked overlap between dumpers and non-dumpers.

DISCUSSION

The ideal test meal should be followed by symptoms which in nature, severity and duration are similar to symptoms occurring after ordinary meals. In gastrectomized patients without spontaneous dumping the administration of the test meal should not give rise to dumping symptoms. In some of my patients the symptoms during the test were characterized as being of exactly the same nature, severity and duration as the usual attacks. The test meal, however was quite different from ordinary meals, and the patients' position was not the same. In some patients the dumping reaction varies greatly with the passage of time (9). Therefore a difference between test symptoms and symptoms ascertained through the questionnaire is to be expected.

The definition of the dumping syndrome based upon the clinical diagnostic index is in agreement with the definition used by other authors (5 24, 26, 30)

The changes in hemoglobin concentration proved to be a good indicator in routine dumping examinations, e.g. for screening purposes, when the relative changes in plasma volume provide sufficient information.

The test meal produced a fall in plasma volume

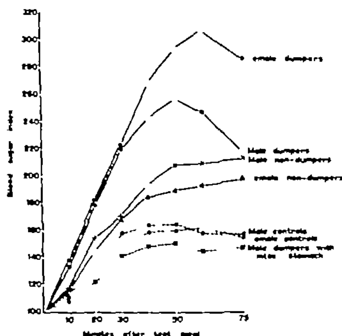


Fig. 7 The mean values of the blood sugar indices. Determinations carried out on 8 female dumpers, 8 female non-dumpers, 18 male dumpers, 15 male non-dumpers, 12 female controls, 12 male controls, and 5 male dumpers with intact stomach.

which was significantly greater in dumpers than in non-dumpers. In many patients the fall in plasma volume is probably an important factor in the production of the dumping syndrome.

The magnitude of the fall in plasma volume, however, was not sufficient to explain the symptoms in all the patients. A small drop in plasma volume was demonstrated in some patients with typical dumping symptoms. Some of these patients were probably very susceptible to a fall in plasma volume on account of a low fasting plasma volume. I have confirmed the observation (22) that some dumpers become almost symptom-free when they gain in weight. This may be due to a decreasing stress caused by the fall in plasma volume in persons with a higher plasma volume. After blood transfusion, symptoms were relieved in dumping patients with low blood volumes (23 27).

On the other hand some patients with a moderate fall in plasma volume were symptom-free. Thus in some patients the development of symptoms may depend upon other factors: the fall in blood volume, the fall in effective circulating blood volume, peripheral vasodilatation, the fall in extracellular fluid, and a decrease in serum potassium concentration. An important factor is probably the individual susceptibility which to some extent determines which patients will develop symptoms (35).

The more rapid and pronounced rise of blood sugar index in dumpers may be due to a defect of carbohydrate metabolism. In many dumpers the previous diet contained little carbohydrate, which may result in a higher degree of hyperglycemia after the test meal (31). Also other factors may explain the high blood sugar rise in dumpers. a low body weight, a great fall in plasma volume, rapid emptying of the gastric remnant, and release of adrenaline.

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TREATMENT OF ESSENTIAL HYPERCHOLESTEROLEMIA WITH CLOFIBRATE AND NICOTINIC ACID

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Abstract. T groups, each f ten patients, with essential hypercholesterolemia (type II) were treated ith alternating clofibrate and nicotinic acid over period of six months. One group started with three months of clofibrate treatment and the other with three months of nicotinic acid treatment. No dietary changes ere made during the test period. All patients responded with reduction of serum total cholesterol and phospholipids after three-month period, either with clofibrate or nicotinic acid. At the end of six months the reduction was more pronounced in both groups, with an average reduction of 28% in serum cholesterol. The effect was independent of which drug was given first.

Essential hypercholesterolemia (type II hyperlipoproteinemia in the classification of Fredrickson et al. (2)) has often a familial occurrence, is characterized by increased amounts of beta-lipo-proteins, total cholesterol and phospholipids in serum, and is associated with a high frequency of early coronary heart disease and xanthomas.

The introduction of lipoprotein electrophoresis in clinical work and the classification of the hyperlipidemias have led to reevaluation of the effect of treatment. So far this type of hyperlipidemia seems to be the most resistant to therapy (2, 7). The effect of different drugs on serum cholesterol in this disease has not yet been settled. Jepson et al. (4) recently reported on the effect of clofibrate and cholestyramine on different types of essential hyperlipidemia. In the present study the effect of clofibrate and nicotinic acid on serum lipids in patients with essential hypercholesterolemia (type II) has been evaluated.

MATERIAL

Ten males and ten females, average age 42 years, with essential hypercholesterolemia were investigated. All of them had Fredrickson type II hyperlipoproteinemia (E.H.C.). Tendinous xanthoma as present in nine cases, xanthelasma in nine, and corneal arcus in fifteen. Plane xanthomas were not seen. Eight patients had symptoms of ischemic heart disease with electrocardiographic abnormalities. Family history gave information of the occurrence of xanthoma, xanthelasma or ischemic heart disease in all patients. One of them probably epresented homozygous case of E.H.C. None of the patients had symptoms of diseases known to cause secondary hypercholesterolemia.

METHODS

The serum lipoproteins were separated by the method of Noble (6), using agar-agarose electrophoresis. The serum level of total and free cholesterol, triglycerides, phospholipids, uric acid and fasting blood sugar were estimated in all patients. Peroral glucose load was carried out in most patients. Blood samples were collected, usually twice, before the commencement of the drug trial, and once at the end of each trial. Lipoproteins were only determined once prior to treatment. Blood was always collected after 14 hours fasting.

No dietary changes were made during the treatment periods. Some patients were on low fat diet and continued on it, the others maintained an ordinary Norwegian diet.

Drugs

The patients were divided into two groups. One group was given daily dose of 2 g clofibrate for 12 weeks and then continued immediately on nicotinic acid 3 g daily for another period of 12 eeks. The other group started with nicotinic acid and continued with clofibrate in the same dosages. Nicotinic acid was administered during the first ten days in dosages starting ith 300 mg, gradually increasing to 3 g daily. This was done to reduce the side-effects with flushing and slight headache usually observed. The clofibrate used was Atromidin, provided by Imperial Chemical Industries.

RESULTS

Before any treatment was started, eighteen of the twenty patients had total cholesterol levels in

Table I. *Mean values of serum lipids, serum uric acid and fasting blood sugar in 20 patients with essential hypercholesterolemia (type II)*

Total cholesterol (mg/100 ml)	Phospholipids (mg/100 ml)	Triglycerides (mg/100 ml)	Free fatty acids (μmol/l)	Uric acid (mg/100 ml)	Fasting blood sugar (mg/100 ml)
490 ± 95[a] (370–830)[b]	358 ± 58 (270–512)	108 ± 21 (64–160)	530 ± 185 (280–900)	4.4 ± 1.0 (2.8–5.3)	78 ± 8.8 (67–97)

[a] Standard deviation. [b] Range.

serum ranging from 370 to 536 mg/100 ml. Between 70 and 80% of the total cholesterol was in the esterified form. The cholesterol/phospholipid ratio showed an average of 1.36 As shown in Table I triglycerides and free fatty acids were within the normal range. All patients had increased amounts of beta-lipoproteins in serum whereas the pre-beta-fraction looked normal. The fasting blood sugar was below 100 mg/100 ml and the response to a peroral carbohydrate load was normal. The serum uric acid levels were within the normal range in all patients.

Initial treatment with nicotinic acid

Table II shows the effect of nicotinic acid and clofibrate on serum lipids in ten patients. The cholesterol and phospholipid levels were reduced in all patients, and the triglyceride level was lowered in eight of the ten patients after nicotinic acid even if they had normal pretreatment values.

Five patients had a reduction of 23% or more of their cholesterol values.

When clofibrate was given an additional reduction of the cholesterol levels was observed in seven patients. There was no correlation between the pretreatment levels of cholesterol and the effect of treatment. At the end of the two treatment periods the mean serum cholesterol was 73% of the pretreatment value, and four had a serum cholesterol less than 300 mg/100 ml. The cholesterol/phospholipid ratio showed no significant changes during the two periods of treatment, and in patients in whom esterified cholesterol was estimated after treatment, no significant changes were observed.

Initial treatment with clofibrate

Table III shows the effect of initial treatment with clofibrate, followed by nicotinic acid, on serum lipid levels in ten other patients. On clofibrate medication all patients showed a reduction of serum cholesterol and serum phospholipids, whereas no significant reduction of the serum triglycerides was obtained. The reduction was less pronounced in these patients than in the group given nicotinic acid initially

Following a period of nicotinic acid, a further reduction of the serum cholesterol was obtained, and a substantial reduction of the serum triglyc-

Table II. *Effect of nicotinic acid and clofibrate in ten patients with essential hypercholesterolemia (type II)*
P = significance of mean values. n.a. = nicotinic acid. cl. = clofibrate

Serum lipids (mg/100 ml)	Nicotinic acid: Pre-treatment mean values	Nicotinic acid: Mean % difference after 12 weeks' treatment	*P* value	Clofibrate: Additional % difference after 12 weeks' treatment	Total mean % difference after 12+12 weeks' treatment with n.a + cl
Total cholesterol	482 (402 780)[a]	−19 (−1 −45)[a]	<0.01	−8 (+26– −26)	−27 (−4 −43)
Phospholipids	351 (270–480)	−20 (−1 −35)	<0.01	−4 (+71 −66)	−24 (−6 – −49)
Triglycerides	99 (64 157)	−15 (+12 −55)	<0.05	−4 (+17– −43)	−19 (+51— −54)

[a] Range.

Table III. *Effect of clofibrate and nicotinic acid in ten patients with essential hypercholesterolemia (type II)*

P = significance of mean % difference. N.S. = not significant. n.a. = nicotinic acid. cl. = clofibrate

Serum lipids (mg/100 ml)	Clofibrate: Pre-treatment mean values	Mean % difference after 12 weeks treatment	*P* value	Nicotinic acid: Additional % difference after 12 weeks treatment	Total % difference after 12 + 12 weeks' treatment with n.a. + cl.
Total cholesterol	498 (370–830)[a]	−15 (−4 — −35)	<0.01	−13 (−1 — −43)	−28
Phospholipids	361 (294–512)	−20 (−6 — −54)	<0.01	−1 (+22 — −11)	−21
Triglycerides	112 (72 –160)	−1 (+114 — −39)	N.S.	−25 (+43 — −89)	−26

[a] Range.

erides occurred. The cholesterol/phospholipid ratio ranged from 1.24 to 1.44 and was not significantly changed by the treatment. At the end of the nicotinic acid period, the mean serum cholesterol value was 353 mg/100 ml, or 72% of the pretreatment value. Three patients had a serum cholesterol level below 300 mg/100 ml.

Side-effects

In five patients originally included in the material nicotinic acid caused troublesome headache and flushing. These patients had to be excluded from the trial. Eighteen of the other twenty patients had slight similar symptoms initially but only eight had these symptoms during the whole treatment period. The symptoms were not so severe that the treatment had to be stopped. No side-effects were observed during clofibrate treatment.

DISCUSSION

A significant reduction of all main serum lipid fractions was obtained in patients with essential hypercholesterolemia, both by nicotinic acid and clofibrate. There was no significant difference in the effect of the two drugs. However the effect of the treatment was more pronounced when the drugs were given alternately with an average reduction of nearly 30% of the serum cholesterol values. This result was observed half a year after the initiation of the treatment, irrespective of which drug was given first. All patients responded to the treatment, also the one who represented homozygous case. Side-effects of the treatment were observed only when nicotinic acid was given. In 20% of the patients these side-effects were so pronounced that this drug was discontinued. These patients were continued on clofibrate only

Dietary treatment of essential hypercholesterolemia (type II) with a diet low in saturated fat and cholesterol, and high in poly-unsaturated fat, is recommended. However the results so far indicate that additional treatment is needed (2, 3 7). A mean reduction in serum cholesterol of 24% may be expected by diet alone (5 9). The present study showed no difference in the effect of the drugs depending on which type of diet the patient used. The exact mode of action of most cholesterol lowering agents is unknown. The studies of Carlson et al. (1) may indicate that nicotinic acid acts by inhibiting the mobilization of free fatty acids from adipose tissue. This is followed by decreased hepatic formation of pre beta-lipoproteins, which again leads to a decreased conversion of pre-beta-lipoproteins to beta-lipoproteins with a subsequent reduction in serum cholesterol and phospholipids. Clofibrate also reduces the level of serum beta-lipo-proteins with an associated reduction of the absolute turnover rates and the extravascular levels of these lipoproteins (8). The studies by Scott and Hurby (8) further indicate a varying response to clofibrate in patients with type II hyperlipoproteinemia, suggesting a subgroup of type II with a more marked reduction in lipoproteins than the majority of these patients.

It thus seems possible that different modes of action may exist for the two cholesterol lowering agents used in the present study. This may ex

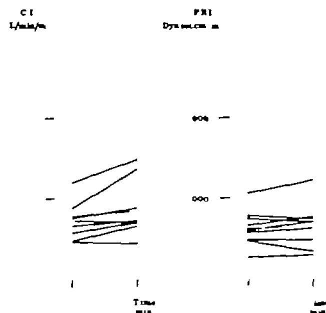


Fig. 5. Effect on cardiac index (CI) and total peripheral resistance index (TPRI) of 25% MVC sustained hand-grip in nine hypertensive patients (group B). Resting values and values at 3 ½ min contraction given.

Cardiac index and total peripheral resistance index

Resting cardiac index ranged from 2.3 to 6.0 l/min/m² (average 3.6 l/min/m²). At 3 ½ min contraction cardiac index ranged from 2. to 7.4 l/min/m² (average 4.3 l/min/m²), i.e. an average increase of 20% (Fig. 5).

Calculated total peripheral resistance index ranged from 1 300 to 5 300 dyn. sec cm⁻⁵m² (average 3 100 dyn. sec cm⁻⁵m²) at rest. At 3 ½ min contraction TPRI ranged from 1 450 to 6 100 dyn. sec cm⁻⁵m² (average 3 200 dyn. sec cm⁻⁵m²) (Fig. 5).

Stroke index

Average stroke index was 43.4 ml/beat/m² at rest and 42.5 ml/beat/m² at 3 ½ min contraction.

Central venous pressure

All patients had normal central venous pressure at rest. During hand-grip there were little or no changes in central venous pressure. In one patient, however central venous pressure rose from 4.5 mmHg at rest to 13.5 mmHg at the end of the contraction with a subsequent gradual fall to 7 mmHg at the end of the observation period.

This patient developed pulmonary oedema within a few hours after the end of the experiment. He was 68 years of age and the oldest patient in the material and was being treated with digitalis for congestive cardiac failure.

DISCUSSION

There was a substantial rise in systolic, diastolic and mean arterial pressure during hand-grip in all patients, most markedly seen during the 50% MVC hand-grip in group A patients, where the average of the mean arterial pressure was extremely high at the end of the contraction. In normotensive subjects, under similar experimental conditions, approximately the same absolute increments in systolic, diastolic and mean arterial pressure have been observed (2, 5 9). However since normotensive subjects start at a lower pressure level, they reach maximal pressures which fall below the maximal pressures reached by our patients.

Contrary to static muscular contraction, dynamic muscular work in normotensive subjects results in an increase in systolic without corresponding increase in diastolic blood pressure, and no or only moderate increase in mean arterial pressure. In hypertensive subjects dynamic work results in a more pronounced increase in mean arterial pressure (1 4 8), but not so marked an increase as found during static muscular contraction in the present investigation.

The pattern of haemodynamic response to static muscular contraction in our hypertensive patients was similar to that in normotensive subjects (3 5 6, 7). Total peripheral resistance, which was found to be higher than normal at rest (8), did not change during sustained hand-grip. The increase in arterial blood pressure was therefore entirely explained by the increase in cardiac out put, which in turn was due to an increase in heart rate, the stroke volume remaining unchanged. When peripheral resistance is constant, a relatively moderate increase in cardiac output will be associated with a corresponding percentage increase in mean arterial blood pressure. However in terms of absolute values this increase in pressure may be large.

The high pressor response to static muscular contraction may have clinical relevance, since static muscular contractions are more common in ordinary daily activity than is perhaps generally realized. They are, for example, involved in all work where objects are being lifted or carried, and in all work with elevated arms. It may well be that sudden rises in mean arterial pressure may b. harmful to patients with hypertension, whose cerebral arteries are often damaged by atherosclerosis and formation of small aneurysms. Furthermore, it is possible that the strain of static muscular contraction by imposing a high pressure load on the heart may provoke acute left ventricular failure.

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RETICULUM CELL SARCOMA IN WALDENSTRÖM'S MACROGLOBULINEMIA AFTER CHLORAMBUCIL TREATMENT

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Abstract. A case of macroglobulinemia Waldenström with very protracted course—in all 17 years, with seven after chlorambucil treatment was started—is presented. The postmortem examination showed changes both of reticulum cell sarcoma and amyloidosis. A review of the literature on macroglobulinemia and reticulum cell sarcoma is given.

Since Waldenström's description of the disease bearing his name, there has been much dispute regarding the nature of the condition. In support of the idea that it is a malignant proliferation of lymphoreticular cells, we may quote reports of a terminal reticulosarcomatous phase in otherwise classical cases. Some of the cases presenting tumorous appearance are, in our opinion, not true examples of sarcoma but merely tumor-like collections of the lymphoreticular cells pertaining to the disease, but no doubt there have been published cases with the combination of Waldenström's disease and reticulum cell sarcoma. Many of the cases, however, are difficult to evaluate because of the different possible combinations of reticulum cell sarcoma and macroglobulinemia. We present a case of clinically well verified Waldenström's disease of 17 years' duration, who developed a rapidly progressive malignant picture after some years of cytostatic treatment. At autopsy a reticulum cell sarcoma-like picture and localized amyloidosis were found.

CASE REPORT

The patient was 1-parous, married woman, born in 1918, who up to 1951 had been quite well. She then began to suffer from recurrent epistaxis. In 1953 proteinuria was diagnosed and at the same time very high ESR, 140 mm/h. Furthermore there was tendency to easy bruising. Later in the same year normochromic anemia was detected. She also suffered from bleeding of the gingiva. In the autumn of 1953 she entered hospital, where during a short time she was treated with urethane because of suspected myelomatosis.

In April 1954 she was for the first time admitted to the Department of Medicine, Malmö General Hospital. At admission the patient had no signs of lymphadenopathy or hepatosplenomegaly. The gingiva was swollen and easily bleeding. A moderate enlargement of the thyroid gland was noticed, but no signs of thyreotoxicosis. She was pale, but her general condition was not bad. The blood pressure was normal.

She had an anemia with hemoglobin of 8.1 g% and red cells 2.3 mill. per mm^3. The leucocyte count was 5 200, with differential count of 66.5% neutrophils, 5% eosinophils, 19% lymphocytes and 9.5% monocytes. The thrombocyte count was 257 000 per mm^3. Serum protein electrophoresis on paper showed distinct and massive monoclonal increase of the gammaglobulin, and the euglobulin reaction (Sia test) was positive. Proteinuria—but Bence Jones protein was not demonstrable. The bleeding time was prolonged to 23 min, but the coagulation time normal. Wassermann reaction negative. Antistreptolysin normal. Sheep cell agglutination test for rheumatoid factor was negative. No cold agglutinins. A sternal marrow aspirate was regarded as normal (Prof. Waldenström).

Ultracentrifugation of the serum proteins revealed 50% as belonging to fraction 18 S, 3% 7 S, and 47% 4.5 S (Ass. Prof. K. O. Pedersen, Uppsala).

On the suspicion that the patient's blood might contain substance with heparinlike qualities, she was treated for short time with protamine sulfate, 200 mg per day and there was possibly some improvement with diminished tendency to epistaxis and gingival bleedings. The patient was thereafter discharged.

In October 1954 she was again admitted because of heavy bleeding from her nose and gingiva. No palpable glands and no hepatosplenomegaly could be detected, but on inspection of her eyegrounds retinal bleedings could be seen. A Bence Jones protein was now found in the urine. The mucosal bleeding decreased this time without further therapy

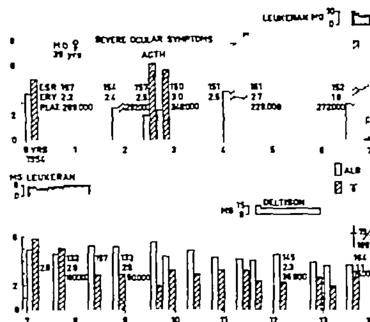


Fig. 1 Diagram showing the course of the patient' disease.

Tw years later in 1956, the patient was again admitted to the Department of Medicine after she had had troublesome gingival bleedings and weak eyesight. She had also had menorrhagiae. She now had an anemia with hemoglobin of 8 g%, but the counts of leucocytes and platelets were normal. In the differential count there were 20% lymphocytes and 13% monocytes, but otherwise nothing remarkable. She had proteinuria, but this time no Bence Jones protein was demonstrable. Her eye grounds showed papilledema and highly engorged veins, and especially in her right eye there were plenty of small round bleedings. The patient evidently suffered from a hyperviscosity syndrome, and the serum viscosity was estimated to be 6.9 at 30° and 5.6 at 37 (much increased). She was given a few blood transfusions (ith regard to her hyperviscosity syndrome w would at present regard this as contraindicated).

During the spring of 1957 further impairment of her eyegrounds occurred, and the gingival bleedings became more evident. A bone marrow biopsy now accorded with the diagnosis of Waldenström macroglobulinemia. Treatment with ACTH was tried but without obvious effect. Later on, in 1959 trial was also made with prednisone in doses of 10–30 mg per day during fh -week period, but no clinical or biochemical result was apparent. In the spring of 1959 the power of sight of her right eye had deteriorated to 0.2 and of her left to 0.5–0.6. A heavy papilledema with protrusion of 4 D and plenteous small bleedings in the eyegrounds were noted. During the next two years the patient condition was unchanged. Since 1954 steady increase of the serum M-component had occurred, from 5.0 g% in 1954 to a maximum of 9.0 g% in April 1961. The total serum protein value had consequently increased from 10.1 g% to 14.7 g%. A Bence Jones protein was still found in the urine. The patient's anemia had also become worse, with hemoglobin values between 4.8 and 6.4 g%. For the first time an estimation of the "background" gamma globulin was made: 0.57 g%, a somewhat low value, which during the following years became still lower at several measurements.

In May 1961 treatment with chlorambucil (Fig. 1) was started in massive doses, usually 4–8 mg per day but during few shorter periods only 1 2 mg per day. With the exception of an interruption between January 1963 and October 1964 this therapy was continued until May 1966, when it was stopped because of low values of leucocytes and platelets. The patient had until then taken total dose of about 4.8 g chlorambucil. The patient tolerated the treatment well, her eyeground findings showed good regression, her general condition improved, her hemoglobin value increased and her M-component was reduced.

After 1966 corticosteroid therapy Deltisone 15–20 mg per day as once again tried but without effect, and furthermore the patient became ery nervous. She had also an anemia with hemoglobin values between 7.3–11.0 g%. The leucocyte count was usually low and she had permanent thrombocytopenia. ESR maximally increased. In October 1967 she was treated in hospital because of mental depression after the death f her mother. At that time she had a hemoglobin alue of 8.0 g%, red cells 2.5 mill. per mm³ 2 400 leucocytes ith a differential count of 58.5% neutrophils, 0.5% eosinophils, 0.5% basophils, 1.5% lymphocytes and 19% monocytes. No signs of increased hemolysis: haptoglobin 130, COHb 0.43%, normal reticulocyte count. The serum electrophoresis now showed its lowest value of the M component, 1.7 g%. Total protein 7.. g% and the "background" gamma globulin also very low 0.37 g%. She had 60 000 platelets per mm³ but a normal titer of all coagulation factors and normal bleeding and coagulation time (Prof. Inga-Marie Nilsson).

In June 1968 she was for the last time admitted to the

Department of Medicine. She had had a swelling of the right leg during the last month, also extending to the lower part of the abdomen. The liver or spleen was not palpable, there were no lymph nodes nor any sign of ascites. Her general condition was rather poor she had pancytopenia with hemoglobin of 4.7 g% 1 000 leucocytes and 16 000 platelets. The bleeding time was now prolonged, 30 min (Duke's method). The platelet adhesivity was normal. A phlebography of her right leg revealed no venous thrombosis, but certain obstacle to the venous outflow in the pelvis was seen. One presumed that the cause could be enlargement of lymph nodes, and radiotherapy in small doses was given against this region. She had several blood transfusions without obvious effect on her anemia. COHb was heavily increased, 1.1% indicating hyperhemolysis, but the reticulocyte count was not elevated. Except for moderate purpura on her legs there was no bleeding tendency. The serum electrophoresis was almost the same as in 1957 with a macroglobulin of 1.9 g%. The patient had moderate fever which was not reduced by antibiotics. All the time her general condition was bad in contrast to her general status in the autumn of 1967. After few weeks she died of large intracerebral hemorrhage.

Autopsy 67 kg/164 cm. Distal parts of both legs edematous with many petechiae. The great vessels in the pelvis and groins passed through thick tumorous masses on the medial aspects of the pelvis. Some lymph nodes were identifiable, but mostly the tumorous tissue was of homogeneous, fleshy appearance. Further down in the groins the lymph nodes were discrete but enlarged, firm and greyish white. The arteries were easily removed, but the veins were heavily infiltrated and compressed to slit like structures. In the anterior mediastinum there was tumor the size of fist, composed of confluent lymph nodes. The thymus was not enlarged. Somewhat enlarged, discrete nodes in the hepatic hilus and along the aorta. Axillary and supraclavicular nodes slightly enlarged, like the spleen (220 g) and the liver (1 750 g).

In the right cerebral hemisphere large hemorrhage. Some small subdural hematomas. Petechiae in the mucous membranes of the gastrointestinal tract. Small perforbital petechiae. Cholecystolithiasis. Heart, lungs and other organs nothing remarkable. Very slight atherosclerosis.

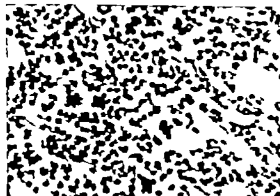


Fig. 2. Autopsy specimen of bone marrow H-E, 320.

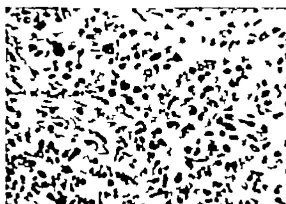


Fig. 3. Pelvic mass. H-E, 255

Microscopically the bone marrow in the vertebrae and femoral shafts was infiltrated with lymphocytoid cells of monomorphous appearance but with many plasmacytoid cells and mastcells (Fig. 2). The lymph nodes in the supradiaphragmatic sites had diffuse internal structure but the sinuses were nowhere completely obliterated. Dense infiltration of lymphocytoid cells in the capsules and surrounding tissues. The tumor-like tissue in the pelvis was of much more polymorphous appearance, and the lymph node structures are mostly destroyed (Fig. 3). Many large, irregular reticulum cells with deeply indented or bilobed nuclei and many mitoses. The veins showed lymphoreticular infiltrates all through, and some of the smaller ones were completely obliterated (Fig. 4). In the walls of the small veins and in the adventitia of the arteries was homogeneous substance, staining pink with eosin, yellow with van Gieson, and metachromatic with stains for amyloid. It was pinkish yellow with Congo-red, with slight green birefringence. The fat cells are surrounded by amyloid rings, and at some points compact deposits were surrounded by giant cells (Fig. 5).

The amyloid substance was seen in the cellular infiltrates in the mediastinum, the axillae and the pelvis and groins, but nowhere in the parenchymatous organs or vessels outside the infiltrations, and not in the bone marrow. Other organs histologically unremarkable. No infiltration in parenchyma or vessels of the brain.

DISCUSSION

Since the first description of the disease, by Waldenström in 1944 which is now generally called primary (essential) macroglobulinemia or Waldenström's macroglobulinemia, several major reviews (10, 11 21 22) have been published and the clinical symptoms and signs are now wellknown.

Our patient had from the outset an anemia and a bleeding diathesis, and also developed serious visual disturbances. The bone marrow cytology



Fig. 4. Tissue from groin. Wall of vein with subintimal lymphoreticular infiltrate and amyloid deposits. H-E, 65.

was typical of Waldenström's macroglobulinemia for many years, with a predominance of lymphoid cells and a considerable admixture of plasma cells and reticulum cells without prominent atypia. In 1961, when our patient's condition was very bad, and the serum macroglobulin had reached its highest value, 9.0 g%, it was evident that some effective treatment was necessary. A course of chlorambucil was started, as proposed by Waldenström already in 1958 (21). In 1961 also came the first report from the Mayo Clinic by Bayrd et al. (1), later enlarged upon in the excellent review of 1967 (11). The therapeutic effect was reflected in reduction of both the anemia and the gamma globulin.

In our patient, too, we got a good clinical response to treatment and an excellent reduction of the macroglobulin level. During the treatment period her anemia improved and she required no transfusions. Her general condition also improved very much and the severe eyeground changes disappeared. However, in spite of the evident reduction of the macroglobulin, which was also maintained for seven years, suddenly and quite unexpectedly a rapid downhill course supervened. At autopsy a distinctly unusual picture was found. The picture in the bone marrow of the vertebrae and femoral shaft was still in accordance with the diagnosis, and some of the lymph nodes in the supradiaphragmatic sites had also a predominance of lymphocytoid cells and the ordinary picture of Waldenström's macroglobulinemia as described, e.g., by Dutcher and Fahey (4). The sarcoma-like tissue on the pelvic walls and in the inguinal regions was of an outright malignant-looking type coming close to reticulosarcomatosis. The patient was given small doses of radiotherapy 420 r cutaneously in four sessions two weeks before death. This might be suspected to have changed the histological picture, but the same type of tissue was seen far from the irradiated field, making this less probable.

Contrary to its near "relative" the myelomatosis, it is not easy to label the primary macroglobulinemia as a definitely malignant disease. The most troublesome symptoms seem to be directly caused by the circulating macroglobulin with blood hyperviscosity (20) and occasionally by the presence of a cryoglobulin (15). A certain degree of relationship to the lymphoproliferative disorders, lymphatic leukemia and lymphosarcoma, can be traced. McKay et al. (12) for instance, propose that the macroglobulinemias actually are a form of lymphosarcoma. Others think that primary macroglobulinemia is a more distinctly limited disease. In isolated cases of lymphatic leukemia a monoclonal gamma-M is found, but here, too, monoclonal gamma-G is not rare (6). Most of the cases with an evident clinical form of lymphatic leukemia or lymphosarcoma combined with a monoclonal macroglobulin have otherwise been classified as secondary macroglobulinemia and not as a disease sui generis. Conclusively one may perhaps say that primary macroglobulinemia is a neoplastic disease with some connection with the lymphatic leukemia-lymphosarcoma group but usually of a low malignancy (see Waldenström (21)).

The reports of reticulum cell sarcoma with macroglobulinemia are, on the other hand, sparse.

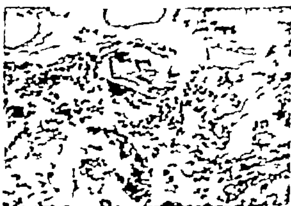


Fig. 5. Amyloid deposit surrounded by foreign body type of giant cell. Kongo red, 260.

Table I. *Cases of macroglobulinemia with reticulum cell sarcoma (not treated with alkylating agents)*

Reference	Comments
Schaub, 1952	Case 2. "Zeta" hyperglobulinemia, 15.4 S at ultracentrifugation. Autopsy: lymphoadenöses reticulosarkom
Zollinger, 1958	Case 8
Creyssel et al. 1959	Reticulum cell sarcoma + macroglobulin (cryomacroglobulin?). Gammaglobulin 1 g %, 17 S at ultracentrifugation
Dutcher & Fahey 1959	Reticulum cell sarcoma of the brain. Bone marrow, spleen and lymph nodes agree with the picture of primary macroglobulinemia. γ-globulin 3.5 g %
Waldenström, 1965	Man, born in 1870. In 1957 several bouts of bronchopneumonia. In 1958 gastrointestinal bleeding and progressive cachexia. γ-globulin 1.5 g %. Autopsy: reticulum cell sarcoma in retroperitoneal lymph nodes and spleen
Waldenström, 1965	Man, born in 1900. Since 1953 anemia perniciosa. In 1957 hyperglobulinemia and sharp band in β-position was detected (max. 1.4 g %). Ad mortem in 1960. Autopsy: pleomorphic malignant lymphoma in lymph nodes, spleen and ventricle
Waldenström, 1965	Woman, born in 1869. Admitted to hospital because of fever and weakness. Anemia and leucopenia. Rapidly progressive cachexia. Sharp band of 0.9 g % in γ-position. Ad mortem in 1960. Autopsy: widespread reticulosarcomatosis
Waldenström, unpublished	Man, born in 1895. Formerly operated on because of neurinoma and parathyroid adenoma. In 1963 suspected of having carcinoma of the stomach. Operation not performed because of his bad general condition. Sharp band of 0.6 g % in β_2-position. Pos. SFBK and acryl fixation test without clinical signs of rheumatoid arthritis. Autopsy: widespread reticulosarcomatosis and renal carcinoma
Kilbinder et al., 1967	Reticulum cell sarcoma with gamma-M max. 1.5 g %. Soon before death treated with nitrogen mustard
Miller 1967	Case 5. E.P. Cold agglutinin disease with cryomacroglobulinemia (γ-globulin 2 g %) with probably simultaneous onset of reticulum cell sarcoma

There have been five communications (3, 4, 7, 16, 28) of solitary cases (Table I). In a paper by Waldenström (22), no less than three cases with the two diagnoses were presented from the Malmö Clinic, and there is also an unpublished case (Waldenström, personal communication). Seligmann and Basch (18) reported three cases in 1968 but there was no information of any treatment or other details. Since therapy with alkylating agents has become more common, cases of macroglobulinemia which have shown signs of reticulum cell sarcoma seem to have been reported somewhat more frequently (Table II). McCallister et al. (11) have two cases: no. 10 who showed a "malignant lymphosarcomatous transformation" and no. 11 with an "anaplastic reticulum cell infiltrate of several organs". Wanebo and Clarkson' patient (24) is of interest among other things because he at first was interpreted as a leukemia. Recently Klemm et al. (8) have published three cases of primary macroglobulinemia treated with alkylating agents, two of whom developed lymphosarcoma and one reticulum cell sarcoma. All cases referred to have had some therapy followed by a rapid downhill course and exitus.

The chronic cold hemagglutinin disease (CCHD), which has been extensively described by Schubothe (17) and in which a tendency to lymphoid proliferation is observed, thereby showing its relationship to primary macroglobulinemia, has also in several cases been treated with alkylating agents, especially chlorambucil. In a report (27) of three cases of CCHD treated with chlorambucil, one of them had expired with a reticulum cell sarcoma. Miller (13) has also reported on a case with cold agglutinin disease and (cryo)-macroglobulinemia, but with a probably simultaneous onset of reticulum cell sarcoma and with only a five-month course until death.

In a paper by Wood and Frenkel (25) a quite typical case of primary macroglobulinemia is presented who, after a transient treatment with cyclophosphamide, developed a lymphosarcoma (simultaneously with a decrease of the macroglobulin). In this case it is more doubtful whether the treatment was of any importance for the course. Moreover Waldenström (23) presented in 1966 a case (G. S.), a woman, who in 1963 had a diagnosis of chronic lymphatic leukemia. In 1964 a monoclonal macroglobulin was detected (max. 2.4 g %). This patient also had Bence Jones proteinuria and osteolytic bone changes. Chlorambucil was given (Table II).

Sarcomatous changes in longstanding cases of

Table II. *Cases of macroglobulinemia treated with alkylating agents with development into reticulum cell sarcoma or closely allied conditions*

Reference	Therapy	Duration from start of therapy until death (y.)	Comments
Wanebo & Clarkson, 1965	Chlorambucil 1 1 1.2 g	2½	During a short time also treated with 6-mercaptopurine. X-ray treatment of the spleen was also given twice. Autopsy: widespread reticulum cell sarcoma
Waldenström, 1966	Chlorambucil 1 4 g	1 9/12	Chronic lymphatic leukemia and increasing monoclonal macroglobulinemia (max. 2.4 g%). Autopsy: enlarged lymph nodes as in chronic lymphatic leukemia, in some reticulum cell sarcoma
McCallister et al., 1967 Case 10	Chlorambucil about 5.9 g	Near 3	Autopsy: extensive malignant sarcomatous transformation of the bone marrow
Case 11	Chlorambucil about 9.6 g	5½	Autopsy: anaplastic reticulum cell infiltrate
Worlledge et al., 1968	Chlorambucil about 1 1 g Cyclophosphamide	1½	Chronic cold hemagglutinin disease. Initial good response to chlorambucil. Azathioprine also tried. Autopsy: widespread undifferentiated reticulum cell sarcoma
Klemm et al., 1968. Case 1	Cyclophosphamide c. 106 g	About 5½	After 2½ years treatment for macroglobulinemia with 34 g cyclophosphamide lymphoreticular sarcoma of parotis developed that totally regressed with local X-ray therapy. Further therapy with cyclophosphamide until death in pneumonia. Hepatosplenomegaly. Spleen biopsy: atypical reticulum cells. N autopsy
Case 3	Cyclophosphamide 156 g	8	Autopsy: massive infiltration by reticulum cell sarcoma
Case 4	Chlorambucil 1630 mg Cyclophosphamide 43 g Melphalan about 200 mg	Over 3	Treated for primary macroglobulinemia. Soon before death development of multiple osteolytic defects (sternal spinal: atypical plasma cells) and tumors of palate and gum with a histological picture of lymphoreticular sarcoma. No autopsy
Pronk et al., 1969 Case 1	Chlorambucil about 2.8 g	2 3/12	After more than 2 years of treatment inguinal lymph node enlargement. Cytological examination: reticulum cell sarcoma. Death of pulmonary embolism
Own case	Chlorambucil 4.8 g	7	See text

chronic lymphatic leukemia have been described, e.g. by Lortholary et al. (9) A similar development seems to be a possibility in chronic myelocytic leukemia (19). Change in an anaplastic direction may also occur in multiple myeloma (e.g. 8) and may possibly be inherent in the nature of Waldenström's macroglobulinemia too.

As Dutcher and Fahey (4) have pointed out, the histological picture of "uncomplicated" cases of macroglobulinemia is apt to be judged as malignant lymphoma, making critical evaluation of the frequency very difficult. The difficulty is enhanced by the cases of lymphoma with secondary macroglobulinemia, where the temporal relationship between the two phenomena is uncertain. Klemm et al. (8) think that the cytostatic treatment has *something to do with the malignant* change, and propose some possible mechanisms:

1 A carcinogenic effect of the alkylating agent.

2. A "natural" development of the disease, in itself having a tendency to develop in a sarcomatous direction. This may be given a better chance of showing up by the prolongation of the life of the patient by treatment.

3 Selection of therapy-resistant clones of cells from the original cellular outfit.

In multiple myeloma, the first possibility seems less probable, as the "secondary" proliferation still has a morphological similarity to the original one though more anaplastic (e.g. 18) A carcinogenic action on an "in itself" malignant cell seems a bit far fetched. Nor does the histological picture in the present case of macroglobulinemia seem to favor this hypothesis. There were many parts with a structure intermediate between the "classical" picture and the frankly sarcomatous one. This may possibly be regarded as support for the theory of a spontaneous evolution from macro-

globulinemia to sarcoma. The third assumption does not seem very attractive in view of the fact that large anaplastic reticulum cells are usually not seen in "classical" macroglobulinemia. The picture is very similar to several of the cases referred to by Lortholary in chronic lymphatic leukemia (9), in which the transformation seems to have occurred in untreated cases, too. To confirm the second assumption, more publications on cases with long survival without treatment would be helpful, but they are difficult to find. One may here emphasize that practically all of the lymphoproliferative and also the myeloproliferative disorders on rare occasions exhibit conversion into each other.

Attention should also be paid to the increasing number of reports on the development of reticulum cell sarcoma in cases of renal transplantation, who during a fairly long time have been treated by extensive immunosuppression, e.g. azathioprine and prednisone, and also with antilymphocytic globulin. Woodruff has recently reviewed the complication of immunosuppression (26) and also the risk of neoplasia. The problem complex has in later years been elucidated in animal experiments, where malignant lymphomas (including reticulum cell sarcoma) can be generated by means of different immunosuppressive methods. In animals oncogenic viruses appear to play a great role that is not yet proven in human medicine.

The doses of cytostatic agents used at transplantation are often larger than is used in the lymphoproliferative disorders. Yet we have to presume that in the latter diseases (including macroglobulinemia) the immune system is already primarily affected and therefore perhaps more sensitive.

However unanimous reports seem to reveal that alkylating agents, especially chlorambucil, are the therapy of choice in macroglobulinemia. We are of the opinion that the chlorambucil therapy gave our patient several years of fairly tolerable life, and the treatment had a striking effect especially on her eyegrounds. Because the pathological cells usually have a rather low number of mitoses, one cannot expect any rapid action from the therapy which instead ought to be quite prolonged. It is probably of great value if the therapy could be maintained to prevent relapses.

The coexistence of Waldenström's macroglobulinemia and amyloidosis has been referred to in the literature only infrequently (Forget et al. (5)). These authors report a knowledge of 11 cases, but more are referred to without extensive data, and the occurrence seems to be more common than mere chance would predict. The amyloid may occur in the "primary" or "secondary" sites. We shall not here discuss the implications of the coexistence. Treatment with cytostatic compounds may precipitate amyloid development experimentally but has evidently not been given in all cases published. It thus seems that amyloid deposition is a possibility belonging to the nature of Waldenström's macroglobulinemia as it is in myelomatosis.

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TUBULAR REABSORPTION OF CALCIUM IN THE DIFFERENTIAL DIAGNOSIS OF HYPERCALCAEMIA

Further Experience

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Abstract. Tubular reabsorption of calcium (TRCa%) has been found to be *increased* in 49 out of 56 patients with hyperparathyroidism (HPT) and in one patient with pseudohyperparathyroidism (P-HPT) (88%), while it was *reduced* in 17 out of 19 patients with non-parathyroid hypercalcaemia (N-PTH) (89%). It seems likely that the diagnostic failures in the HPT group may be due to an excessive intake of calcium on free diet (4), erroneous creatinine analysis (2), and combination of HPT with sarcoidosis *disturbing the calcium metabolism* (1). Within the N-PTH group one diagnostic failure on free diet could not be reproduced on standard diet. The other failure occurred in *smoking alkalosis*, in which the hypercalcaemia was due to the very fact that alkalosis imitated the action of parathyroid hormone upon the TRCa%. Among the traditional diagnostic procedures—serum phosphate, alkaline phosphatases, phosphate excretion index, and urinary excretion of calcium—the last one is the most valuable. Forty-seven per cent of patients with HPT had *normocalciuria*, while this applied to only one out of 19 in the N-PTH group. It is concluded that determination of TRCa%—*performed on standard diet*—is well-suited for distinguishing HPT and P-HPT from N-PTH. The method cannot distinguish between HPT and P-HPT and it may fail if HPT is combined with another cause of hypercalcaemia or if hypercalcaemia is due to non-parathyroid factor acting *direct* upon the TRCa%. Calculation of the "TRCa %—by substituting the concentration of ultrafiltrable calcium by *total calcium*—gives the same qualitative separation between the groups as TRCa%.

Classification of disturbances of calcium metabolism according to endocrinological principles presupposes a knowledge of the concentrations of calcium and parathyroid hormone in the serum. The *first* step is to ... patient has hyper normo- or ... severe disturbances it is enou... total calcium concentration ... borderline cases determinat... tion of ultrafiltrable calciu... of ionized calcium (Ca^{++}) ... able (8, 12, 18 19 21). If ... detected, the *next* step shou... whether the secretion of parath...roid increased or decreased. Radioimmunoassay for parathyroid hormone is the method of choice, but unfortunately the facilities for this procedure are not generally available. Instead, resort m...t be had to indirect methods, which rec...rd th... effects of the parathyroid hormone upon th... renal tubules or to the cortisone suppression test (4, 5).

Parathyroid hormone acts upon the renal tubules by reducing the reabsorption of phosphate and increasing that of glucose and calcium. The former is inapplicable for differential diagnostic purposes (18) while the maximum tubular reabsorption capacity for glucose (TmG/GFR) has certain advantages and limitations as pointed out by Halver (9). In previous studies we introduced determination of the tubular reabsorption of calcium (TRCa%) for differential diagnostic use in hypercalcaemia (16–18). The present publication presents our experience in a large material of hypercalcaemic patients.

MATERIAL

The material comprises 76 patients with an elevated mean concentration of Ca^{++} and for whom simultaneous

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Table I *Data for use in calculating and interpreting the tubular reabsorption of calcium (TRCa % and ^a TRCa %) and the phosphate excretion index (PEI) in 76 patients with hypercalcaemia)*

Pat. no.	Initials	Sex	Age	Diet[a] Ca (mg/24 h)	Diet[a] P (mg/24 h)	Blood TOCa (mg/100 ml)	UFCa (mg/100 ml)	Ca^{++} (mg/100 ml)	P (mg/100 ml)	A.Ph.[b] (U/l)	St. bic. (mEq/l)	pH
Normal range						9.2–10.6	6.55–7.65	6.00–6.60	2.5–4.6	13–38	21.3–25.8	7.36–7.42
Primary hyperparathyroidism												
1	AHJ	♀	48	*917*	*1055*	17.2	13.03	11.85	2.6	35	25.4	7.47
2	IT	♂	53	—	—	12.8	9.80	—	1.7	40	24.5	7.41
3	KEO	♀	37	—	—	11.7	8.95	8.15	2.4	55	20.7	7.36
4	BSH	♀	24	—	—	12.2	8.50	7.25	3.0	32	22.4	7.42
5	GP	♂	51	—	—	11.6	8.50	7.75	2.3	29	23.4	7.42
6	KJ	♂	55	*705*	*1173*	11.4	8.60	7.55	3.3	24	23.0	7.40
7	JAN	♂	24	*941*	*1180*	11.2	8.50	7.55	2.8	66	24.0	7.41
8	AMS	♀	56	—	—	11.1	8.32	7.10	3.3	35	25.1	7.44
9	NKC	♀	62	*724*	*1121*	11.0	8.35	7.45	2.9	93	23.8	7.39
10	MH	♂	48	—	—	11.1	7.88	6.98	2.9	29	24.8	7.39
11	EE	♀	58	—	—	10.6	7.55	6.70	2.8	29	25.5	7.44
12	PVN	♂	43	898	1333	10.6	7.83	—	2.8	45	23.2	7.39
13	PJ	♂	59	1209	1175	10.5	7.70	6.88	2.8	49	22.0	7.38
14	ALJ	♂	53	—	—	10.4	7.95	6.95	3.5	43	21.3	7.37
15	EC	♂	53	—	—	16.5	12.00	10.40	2.0	60	21.6	7.38
16	CWJ	♂	51	*793*	*1070*	15.0	11.36	9.93	3.1	190	23.2	7.42
17	GMP	♀	57	—	—	14.7	11.45	9.90	3.5	156	21.7	7.45
18	EMC	♀	47	818	1047	14.8	11.60	10.40	1.9	36	—	—
19	AHO	♂	52	*810*	1139	14.2	11.00	10.13	2.2	62	24.0	7.43
20	EJP	♀	41	*804*	*1051*	14.8	11.93	10.75	2.1	44	22.6	7.35
21	GL	♀	55	795	1079	14.0	10.57	8.90	2.5	36	21.7	7.39
22	SP	♀	54	—	—	12.4	8.95	—	2.9	—	—	—
23	PCBB	♂	45	—	—	1[illegible].1	9.15	8.00	2.5	42	25.4	7.39
24	EKJ	♀	56	—	—	11.6	8.80	7.85	2.4	24	22.5	7.42
	JM	♂	29	*864*	*1200*	11.6	8.75	7.95	2.8	39	25.2	7.41
26	CES	♀	55	—	—	11.4	8.40	7.60	3.5	40	24.0	7.42
27	KMS	♀	60	—	—	11.4	8.55	7.60	3.2	34	—	—
28	EHA	♀	44	808	1051	11.8	8.70	—	2.4	29	23.3	7.38
29	EAM	♀	48	796	1021	11.4	8.60	7.50	2.8	32	22.9	7.38
30	VHKM	♀	64	—	—	11.2	8.70	7.40	2.8	48	24.5	7.41
31	ECWJ	♂	47	*746*	*838*	11.3	8.83	7.88	2.6	39	25.1	7.40
32	AR	♀	29	—	—	11.1	8.20	7.60	—	—	—	7.38
33	IWN	♀	76	—	—	10.8	8.35	7.50	2.7	34	21.2	7.38
34	HH	♂	72	—	—	9.9	7.38	6.70	3.3	64	23.7	7.38
35	KMKL	♀	54	—	—	11.0	8.10	—	2.5	25	23.3	7.44
36	KRC	♀	49	—	—	16.4	12.70	—	2.6	59	21.0	7.36
37	EAMJ	♀	47	833	1120	11.1	8.45	7.40	2.9	18	21.5	7.39
38	PS	♂	59	776	1074	12.0	8.75	8.10	2.4	42	19.6	7.32
39	KOS	♂	50	799	1069	12.5	9.40	8.08	2.2	29	20.7	7.37
40	KAEJ	♀	63	*807*	1116	10.8	7.90	6.97	4.0	20	19.9	7.33
41	NWL	♀	67	*777*	991	14.5	11.15	9.98	2.3	38	20.7	7.38
42	BL–M	♂	46	*734*	1171	11.4	8.70	7.95	3.3	22	23.0	7.41
43	HST	♀	38	*885*	1101	11.6	8.30	7.75	3.0	33	22.7	7.38
44	PJMSK	♀	64	—	—	10.5	7.50	7.00	2.7	34	23.0	7.35
44b	—	—	—	*759*	1209	11.0	7.63	7.17	3.1	50	23.9	7.37
45	CMF	♀	57	648	1005	12.1	8.90	—	2.8	63	22.7	7.39
46	BA	♀	18	755	887	10.9	7.60	7.08	3.5	22	21.7	7.35
47	JAH	♂	48	813	1148	11.7	8.75	7.38	2.9	32	21.8	7.36
48	HHA	♂	50	775	1386	11.1	8.60	7.83	2.6	58	21.5	7.35
49	VPS	♂	45	—	—	10.2	7.65	6.75	2.9	30	23.9	7.40
49b	—	—	—	<150	—	10.8	8.05	6.95	3.1	30	26.6	7.47
49	—	—	—	—	—	9.8	7.30	—	3.3	33	—	—
49d	—	—	—	<150	—	—	—	—	—	—	—	—
Hyperparathyroidism associated with sarcoidosis												
50a	MEJ	♀	67	*1061*	*1321*	12.9	9.85	8.95	2.8	—	—	—
50b	—	—	—	*901*	*1156*	12.0	9.30	8.35	3.0	40	22.5	7.39
51a	BES	♀	23	*870*	*1096*	12.5	9.77	8.44	2.7	20	26.3	7.47
51b	—	—	—	*870*	*1096*	10.5	8.45	7.40	2.6	17	26.9	7.51
51	—	—	—	*724*	*1121*	9.8	*7.35*	6.30	3.5	30	*23.5*	7.44

Urine CCr (ml/min)	Ca (mg/24 h)	TRCa (%)	*TRCa (%)	24-h-P.E.I. −0.08–+0.09	Na (mEq/24 h)	No. of days	Comments
43	366	95.4	96.5	+0.30	45	2	
82	208	98.2	98.6	+0.31	—	1	
97	277	97.8	98.3	+0.20	131	2	
83	153	98.5	99.0	0.00	—	1	
03	440	96.4	97.4	+0.21	144	3	
100	366	97.1	97.8	+0.09	—	1	
89	160	98.6	98.9	+0.20	74	2	
78	344	96.2	97.2	+0.07	99	4	
83	163	98.4	98.8	+0.09	61	3	
47	492	97.1	97.9	+0.10	—	3	
85	190	98.0	98.5	+0.06	—	2	
29	285	98.0	98.6	+0.15	90	4	
93	234	97.7	98.3	+0.09	38	3	
82	218	97.0	97.7	+0.05	97	4	
37	370	94.3	95.8	+0.48	75	1	
38	149	97.7	98.2	+0.36	57	8	
18	118	96.1	97.0	+0.49	—	1	
98	487	97.0	97.7	+0.23	79	2	
79	474	96.3	97.1	+0.35	54	2	
74	307	97.6	98.1	+0.34	74	4	
79	408	96.6	97.5	+0.20	62	4	
77	172	98.3	98.8	+0.04	90	1	
95	506	96.0	96.9	+0.14	56	2	
71	105	98.8	99.1	+0.20	—	1	
152	514	97.3	97.9	+0.10	—	3	
86	541	94.8	96.2	+0.07	78	2	
80	305	96.9	97.7	+0.02	—	1	
	417	97.3	98.0	+0.16	68	1	
77	417	98.1	98.5	+0.04	56	3	
73	461	94.9	96.1	+0.14	—	2	
74	66	99.3	99.5	+0.08	—	3	
89	219	97.3	98.0	—	—	1	
82	289	97.0	97.7	+0.12	68	5	
[illegible]	123	97.8	98.2	+0.15	—	3	
[illegible]	364	97.5	98.1	+0.14	—	2	
	503	95.2	96.3	+0.24	66	1	
	298	97.6	98.2	+0.10	47	4	
96	311	97.6	98.3	+0.16	67	3	
	295	97.7	98.3	+0.22	66	5	
	36	98.4	98.9	+0.37	77	5	
[illegible]	193	96.9	97.6	+0.37	60	4	
	309	97.7	98.2	+0.07	39	2	
	303	97.2	98.0	+0.05	53	6	
6	295	97.7	98.3	+0.11	84	1	
	297	96.2	96.7	+0.11	66	4	4 mo later
	362	97.2	97.9	+0.10	67	2	
	103	99.1	99.4	−0.01	108	3	
	213	98.3	98.8	+0.12	56	3	
16	172	98.8	99.1	+0.21	37	2	
	365	95.7	96.8	+0.12	—	2	
[illegible]	271	97.8	98.3	+0.06	—	2	Low Ca diet 5th and 6th day
18	381	96.9	97.7	+0.05	—	1	42nd day after PTX
	142	—	—	—	—	2	Low Ca diet 4th and 5th day
3	290	97.2	97.9	+0.19	—	2	Prednisone, 15 mg, 17th and 18th day
[illegible]	178	97.9	98.3	+0.18	63	3	6 mo. later
	356	94.9	96.0	+0.38	72	9	
[illegible]	176	98.0	98.4	+0.20	—	2	Cortisone, 150 mg, 11th 12th day
	134	98.0	98.5	+0.06	29	2	3 mo. after PTX

Table I (cont.)

Pat. no.	Ini-tials	Sex	Age	Diet[a] Ca (mg/24 h)	Diet[a] P (mg/24 h)	Blood TOCa (mg/100 ml)	UFCa (mg/100 ml)	Ca^{++} (mg/100 ml)	P (mg/100 ml)	A.Ph.[b] (U/l)	St. bic. (mEq/l)	pH
Normal range						9.2-10.6	6.55-7.65	6.00-6.60	2.5-4.6	13-38	21.3-25.8	7.36-7.42
Hyperparathyroidism associated with renal tubular acidosis												
52a	ECMH	♀	44	1201	1423	10.4	7.95	6.80	3.4	21	16.3	7.30
52b	—	—	—	1147	1249	11.4	8.85	7.35	2.8	17	28.3	7.49
52	—	♀	48	745	1171	10.9	7.88	—	3.0	23	21.6	7.35
Hyperparathyroidism associated with mastocytosis												
53	KAM	♀	55	—	—	10.6	7.98	7.18	3.0	—	21.5	7.36
53b	—	—	—	867	1044	11.0	7.83	7.00	3.2	36	22.0	7.37
Hyperparathyroidism associated with neurofibromatosis (von Recklinghausen)												
54	THS	♂	30	740	1087	10.8	7.80	6.75	4.5	35	22.3	7.37
Hyperparathyroidism associated with nephrosis (chronic glomerulonephritis)												
55	ALE	♀	55	731	1040	10.3	8.30	7.80	4.3	19	22.3	7.40
Hyperparathyroidism associated with rheumatoid arthritis in long-term treatment by prednisone, calcium phosphate and vitamin D												
56	EW	♂	51	2452	2416	10.8	8.40	7.63	4.7	32	17.7	7.33
Pseudohyperparathyroidism due to oat-cell carcinoma of the pancreas												
57	AAM	♂	72	—	—	10.7	8.02	7.03	3.3	71	22.1	7.35
Poststrumectomy hypoparathyroidism overdosed by vitamin D												
58	PE O	♂	45	784	1094	11.6	8.85	7.60	4.2	24	28.9	7.48
59	EB	♀	51	778	1156	11.3	8.28	7.13	3.0	19	24.3	7.47
60	MKH	♀	64	—	—	11.6	9.15	8.05	3.0	26	—	—
Ordinary hypercalcaemic sarcoidosis												
61	EVK	♀	58	—	—	13.0	10.05	8.25	3.3	24	23.6	7.39
61b	—	—	—	839	1098	11.2	8.90	7.41	3.5	26	23.2	7.39
62	ABH	♂	69	—	—	14.5	11.42[c]	—	3.7	—	—	—
63	KMC	♂	33	—	—	13.6	10.88	9.55	4.4	28	26.4	7.43
63b	—	—	—	—	—	8.9	6.95	6.30	2.7	28	23.9	7.44
64	LKK	♂	25	—	—	10.9	8.90	7.10	2.6	44	24.3	7.43
64b	—	—	—	1094	1179	11.8	9.40	8.06	3.1	41	24.3	7.42
65	TFS	♂	26	—	—	11.3	8.45	7.65	4.2	88	25.1	7.43
65b	—	—	—	705	1173	10.6	8.18	7.25	4.5	68	27.2	7.45
65	—	—	—	790	1172	11.3	8.73	7.48	4.6	50	24.6	7.41
65	—	—	—	790	1172	10.5	7.95	6.80	4.3	44	25.6	7.45
66a	AJC	♂	43	—	—	11.4	8.60	7.70	2.7	30	21.6	7.41
66b	—	—	—	—	—	9.8	7.40	6.55	2.7	24	22.5	7.39
67a	TOHP	♂	25	—	—	13.2	9.20	—	3.0	77	24.1	7.42
67b	—	—	—	—	—	10.0	7.40	6.35	2.1	—	25.9	7.46
68	TSH	♀	57	—	—	11.8	9.30[c]	7.95	2.9	30	24.3	7.36
69	HB	♀	13	791	1100	12.3	9.30	7.98	3.2	39	22.0	7.38
69b	—	—	—	—	—	10.1	7.70	7.15	2.6	39	23.6	7.37
Remittent hypercalcaemia of uncertain origin, probably due to collagen disease												
70a	EMKR	♀	56	883	1208	11.9	9.45	7.95	3.6	28	21.4	7.39
70a	—	—	—	883	1208	9.9	7.95	7.10	3.3	24	21.5	7.36
70a	—	—	—	883	1208	9.0	7.45	6.60	3.4	24	20.5	7.38
71	SKI	♂	61	749	1213	11.0	8.60	7.35	4.6	35	23.3	7.40
71b	—	—	—	749	1213	10.9	7.40	6.30	4.1	30	21.7	7.46
71	—	♂	62	779	1175	10.1	7.70	6.80	3.7	42	23.9	7.36
Malignant lymphomas												
72a	BSH	♂	28	—	—	9.9	7.50	6.60	3.6	46	23.6	7.47
72b	—	—	—	—	—	13.4	9.45	7.93	3.9	40	25.3	7.41
73	AFL	♂	62	795	1057	14.6	11.10	10.38	3.1	85	23.3	7.43

Cr (ml/min)	Ca (mg/24 h)	TRCa (%)	*TRCa (%)	24-h-P.E.I. −0.09–+0.09	N (mEq/24 h)	No. of day	Comm
74	147	98.2	98.7	+0.10	107	5	Spontan
79	73	99.3	99.4	+0.15	129	4	Overdo
45	104	98.6	99.0	+0.17	136	3	Well-co
13	412	96.8	97.5	+0.10	—	3	
73	342	95.9	97.0	+0.11	30	6	5 mo
17	199	97.9	98.5	−0.06	54	5	
40	42	99.1	99.3	+0.11	31	4	
33	40	97.1	97.7	+0.39	65	5	Vitam Predn
41	67	99.0	99.3	+0.25	121	4	
23	361	87.9	90.9	+0.45	—	8	Vitamin D [illegible] ped bef re invest.
37	384	91.2	93.5	+0.45	75	4	Vitamin D stopped be ore invest.
29	456	88.0	90.5	+0.42	—	2	Vitamin D stopped bef re invest.
34	779	84.0	87.7	+0.53	104	1	
31	389	85.8	88.7	+0.46	75	11	
30	781	84.0	87.4	+0.45	—	1	
33	517	90.1	92.1	+0.28	61	4	Anorexia and vomiting
72	276	96.2	97.0	+0.23	—	2	Prednisone, 22nd and 23rd day
14	542	94.2	96.9	—	—	1	
84	540	95.3	96.2	+0.13	—	8	
48	393	90.0	92.5	+0.21	—	1	
73	339	95.8	96.8	+0.06	33	3	
68	393	95.3	96.4	+0.04	56	4	3 mo. later
76	312	96.4	97.2	+0.04	65	4	
63	733	90.5	92.9	+0.41	129	1	
73	441	95.6	96.1	+0.17	164	2	Cortisone, 150 mg, 12th and 13th day
33	407	91.0	93.6	+0.46	—	2	
75	425	94.7	96.1	+0.32	—	1	Cortisone, 150 mg, 13th day
79	530	89.7	91.9	+0.48	57	2	
37	390	87.9	91.0	+0.38	89	2	
71	335	95.7	96.8	+0.30	83	2	Cortisone, 100 mg, 13th and 14th day
6	443	91.8	93.5	+0.34	80	4	3rd–6th day of study
0	263	95.4	96.5	+0.28	85	2	10th–11th day of study
6	124	98.3	98.6	+0.07	35	2	20th–21st day of study
1	396	94.1	95.4	+0.03	88	2	
7	425	95.4	96.9	+0.02	121	1	Cortisone, 150 mg, 12th day
5	233	98.2	98.6	+0.02	75	4	8 mo. later
5	371	95.4	96.6	+0.08	122	2	
3	365	88.5	91.9	+0.37	110	1	3 mo. later
1	471	94.2	95.6	+0.28	67	3	

Table I (*cont.*)

Pat. no.	Ini-tials	Sex	Age	Diet[a] Ca (mg/24 h)	Diet[a] P (mg/24 h)	Blood TOCa (mg/100 ml)	UFCa (mg/100 ml)	Ca^{++} (mg/100 ml)	P (mg/100 ml)	A.Ph.[b] (U/l)	St. bic. (mEq/l)	pH
Normal range						9.2–10.6	6.55–7.65	6.00–6.60	2.5–4.6	13–38	21.3–25.8	7.36–7.42
Myelomatosis												
74	VO	♂	67	—	—	11.9	10.10	8.90	4.0	44	25.5	7.43
Idiopathic intestinal hyperabsorption hypercalcaemia												
75	HOCN	♂	44	—	—	10.8	7.97	7.08	3.2	33	25.0	7.41
75 *b*	—	—	—	866	*1154*	10.6	8.01	7.27	3.8	29	23.9	7.38
75	—	♂	45	836	*1179*	10.8	8.03	7.28	3.6	34	24.0	7.39
75 *d*	—	—	—	836	*1179*	10.8	7.95	7.10	3.4	48	23.5	7.39
Hypercalcaemia associated with hypokalaemic alkalosis secondary to surreptitious vomiting												
76	SHC	♀	39	<*1177*	<*1314*	10.3	8.43	6.90	4.2	25	32.1	7.46

[a] Calcium and phosphorus contents given in roman letters derive from food tables, while chemical analysis of diets is indicated by the use of italics.
[b] Alkaline phosphatases.
UFCa was *calculated* from TOCa by use of the mean TOCa/UFCa ratio in ordinary hypercalcaemic sarcoidosis (18).
In some subjects the values for CCr, TRCa % and P.E.I. differ slightly from those published previously; this is due to change in the procedure of calculation.

determinations of TRCa% are available (Table I). Parathyroidectomy was carried out in all cases of hyperparathyroidism.

Hyperparathyroidism was diagnosed by excluding other causes of hypercalcaemia, by taking the case-history and by clinical, radiological, and laboratory investigations. In cases where this was not sufficient the indication for ... was supported on ... negative cortisone test ( ), high TmG/GFR (9), or ... high TRCa% (18). It should be stressed that ... low TRCa% never made us decide against an operation (cases 15, 26, 30, 44, 49, 51 and 53). By these preoperative criteria hyperparathyroidism was diagnosed in cases 1–56, and pseudohyperparathyroidism in case 57 (Table I). In 49 patients the diagnosis was supported by histological demonstration of an adenoma or hyperplasia, while in the remaining seven cases it was confirmed by sustained normalization of Ca^{++} after parathyroidectomy. According to our previous studies it seems likely that this functional criterion is more reliable than histological examination in cases of mild hyperparathyroidism (19).

In addition to hyperparathyroidism some of the patients had other diseases ... hich might influence the calcium-phosphorus metabolism (cf. Table I, cases 50–57).

The patients with *hyperparathyroidism and sarcoidosis* were reported previously (8, 9, 11, 20).

In ... patient with *hyperparathyroidism and renal tubular acidosis* the disease began as early as 1938 with severe metabolic acidosis, hypokalaemic paralysis, and nephrocalcinosis as reported by Drewes (6). We have followed this patient since 1963. Her urinary pH invariably exceeded 6.0 and her TOCa was normal or slightly increased. In 1965 ... slightly elevated Ca^{++} was found during spontaneous acidosis (case 52); immediately after the patient received an overdose of sodium bicarbonate which increased the TRCa% and accentuated the hypercalcaemia (case 52 *b*, Fig. 3). In 1968 she exhibited accentuated nephrocalcinosis, mild hypercalcaemia, and ... high TRCa% in spite of *adequate* bicarbonate medication (case 52 *c*, Fig. 3). Parathyroidectomy disclosed one adenoma (370 mg) with ... rim of normal parathyroid tissue beneath the capsule and two normal parathyroid glands weighing 30 and 55 mg. The TOCa fell to 8.7 mg/100 ml.

Another patient with *hyperparathyroidism and mastocytosis* exhibited typical urticaria pigmentosa. X-ray examination did not reveal nephrolithiasis, but showed generalized decalcification of the skeleton and multiple bone cysts, phenomena which may occur in hyperparathyroidism as well as in mastocytosis. Massive mast-cell infiltration was found in skin and bone biopsies. Parathyroidectomy disclosed an adenoma (430 mg) and three parathyroid glands which on biopsy proved normal. Two months postoperatively the UFCa and Ca^{++} ... ere 7.40 and 6.35 mg/100 ml respectively.

In a patient with *hyperparathyroidism and neurofibromatosis* the unusually low phosphate excretion index suggested that in some way or other the neurofibromatosis modified the effect of parathyroid hormone. An increased secretion of growth hormone might be responsible for this finding, but the patient did not exhibit any signs of acromegaly. A negative cortisone test (Fig. 6) indicated parathyroidectomy. The two parathyroid glands removed in this operation differed from the remaining two histologically normal glands in having no fat cells and in presenting only one type of cell. Two months after the operation the Ca^{++} and UFCa were 6.68 and 7.65 mg/100 ml respectively.

Severe steatorrhoea was the initial sign in a patient

Urine

CCr (ml/min)	Ca (mg/24 h)	TRCa (%)	"TRCa (%)	24-h-P.E.I. −0.08–+0.09	N (mEq/24 h)	No. of days	Comments
53	358	95.3	96.1	+0.11	—	2	
121	732	94.5	95.9	+0.08	—	6	
116	616	95.4	96.5	0.00	55	8	6 mo. later
110	518	95.9	97.0	+0.03	—	4	12 mo. later
115	539	95.7	96.9	+0.05	—	2	Cortisone, 150 mg, 13th and 14th day
75	56	99.4	99.5	−0.04	77	6	

his *pseudohyperparathyroidism due to an oat-cell carcinoma of the pancreas*. Generalized decalcification of the skeleton without metastases, hypercalcaemia, high TRCa% and a palpable neck tumour suggested hyperparathyroidism, but *neck exploration disclosed lymph-node metastasis* from an oat-cell carcinoma as well as three histologically normal parathyroid glands. Postoperative lymphography showed metastatic obstruction of the intestinal lymphatic drainage. At autopsy the primary tumour was found in the pancreas, and the fourth parathyroid gland proved normal.

In accordance with the principle of exclusion, the group of *non-parathyroid hypercalcaemia* must comprise the patients in whom another cause of hypercalcaemia is demonstrable (cases 58–76). Various findings militate against *simultaneous* hyperparathyroidism in these patients, viz. first parallel remission of hypercalcaemia and of the basic disease (cases 58, 65, 70 and 71), secondly positive result of cortisone tests (cases 59–64, 66–69 and 71–74, Fig. 7, Table VIII), thirdly reduced TmG/GFR (cases 58–61, 64–65 and 75, cf. previous publications (9, 12, 20)), and fourthly negative neck explorations (cases 62 and 75). One patient (case 72) had a normal TmG/GFR, 2.19 and 2.44 (normal range 1.68–2.44 [mean ± 2S.D. (9)]), but the hypercalcaemia yielded completely to cortisone (Fig. 7, Table VIII). Owing to the tendency to spontaneous remission, it was decided to use *the first set of observations during hypercalcaemia* as a basis for calculation of the mean values for the group (Table III) and for the tabulation of the individual parameters (Tables IV–VII).

Some of the patients with *vitamin D overdosage* and *ordinary hypercalcaemic sarcoidosis* (cases 8 and 61–65) have been reported previously (8, 11, 17, 20). Also a patient with *idiopathic intestinal hyperabsorption hypercalcaemia* was reported (12).

Collagen diseases are not considered among the known causes of hypercalcaemia. Therefore, the two patients thus classified will be described in detail.

Case 70 suffered from intermittent, localized oedema *and rash for ten years. Signs of hypercalcaemia* appeared in July 1967 and in January 1968 she was admitted to Medical Department A suspected of hyperparathyroidism.

The positive findings in the *physical examination* include generalized lymph-node enlargement, mild hepatomegaly and bursitis on the back of the hands. The *X-ray findings* are negative: no *hilar adenitis* or pulmonary infiltrations, no skeletal decalcification, and an i.v. pyelography was normal apart from questionable nephrocalcinosis.

The main *laboratory studies* showed definite hypercalcaemia which receded spontaneously and completely. Haemoglobin 11.9 g/100 ml, ESR 11 mm/h, pronounced *eosinophilia* in the peripheral blood 17,000/μl (42%) which dropped to 3,400/μl as the hypercalcaemia subsided, Coomb's test faintly positive, LE cells 0, moderate hypoalbuminaemia 3.4 g/100 ml, mild hypergammaglobulinaemia 1.4 g/100 ml, negative Mantoux I and II and electromyographic signs of myogenous affection.

Biopsies

Bone marrow: 24% eosinophilic leukocytes. *Lymph-nodes* from axillary and inguinal regions: pronounced reticular cell proliferation. *Liver*: normal. *Kidney*: normal glomeruli, scattered atrophic tubules, and interstitial as well as tubular calcium deposits. *Striated muscle*: severe inflammatory changes with degeneration of muscular fibrils, fibrinoid necroses, and marked perivascular cellular infiltrations without signs of vasculitis, in short, changes *typical of collagen disease*. None of the biopsies

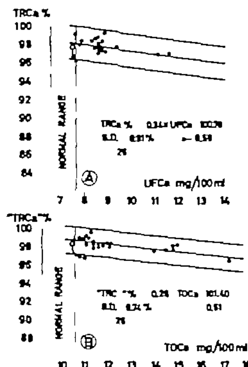


Fig. 1. The tubular reabsorption of calcium (TRCa %) in patients with *hyperparathyroidism* studied on *standard diet* (= 26). TRCa % has been calculated from the means of serum ultrafiltrable calcium, UFCa (A), and "TRCa % from serum total calcium, TOCa (B). arrow connects observations on case no. 44 (see).

showed specific signs of carcinomatosis, myelomatosis, leukaemia, Hodgkin's disease, or sarcoidosis.

In the other patient, case 71, *collagen disease* was diagnosed by exclusion. The patient had history of cervical lymph-node tuberculosis and prepyloric ulcer confirmed by X-ray. His present complaints were periodical epigastric pain, fatigue, muscular weakness, and weight loss of 10–12 kg in the course of 2 years. No consumption of milk, vitamin D or alkali.

Physical examination

An emaciated man looking chronically ill with moderate muscular atrophy but no clinical signs of sarcoidosis, Hodgkin's disease, cancer, or thyrotoxicosis. *Chest radiography* revealed a calcified tuberculous lesion without hilar adenitis or infiltrations, while examination of the skeleton disclosed poor dental status and moderate generalized decalcification. X-ray of the oesophagus, stomach, biliary tract, and colon as well as i.v. pyelography were normal.

Laboratory studies revealed cortisone-sensitive, later spontaneously remitting hypercalcaemia, high normal serum phosphate level, pronounced hypercalciuria, and a highly negative calcium balance (mean of 3 6 days: −674 mg/24 h). There was little else positive: normocytic hypochromic anaemia, 10.7 g/100 ml with a normal serum iron and transferrin, ESR 47 mm/1 h, border line elevation of alkaline phosphatases and glutamic-pyruvic transaminase, mild hypoalbuminaemia, and slightly elevated α_2 and γ-globulin fractions. Mantoux I was positive. Investigations for LE cells, Coombs' test, serum bilirubin, white cell and differential count, microscopic examination and culture of the urine, as well as investigation of the faeces for blood showed normal conditions.

Biopsies

Bone marrow normal. *Mediastinal lymph-node*: caseous necroses without signs of active tuberculosis. *Liver*: mild periportal fibrosis. *Skin*: small non-specific, perivascular cellular infiltrations. *Striated muscle*: normal. None of the biopsies showed any specific signs of carcinomatosis, myelomatosis, leukaemia, Hodgkin's disease, sarcoidosis, or collagen disease.

Course

During three months in hospital the general condition gradually improved, the haemoglobin concentration rose to 12.3 g/100 ml, and the ESR dropped to 28 mm/h. At a follow-up admission eight months later the disturbance of calcium metabolism had remitted (case 71 d), the patient was in good health and had gained weight (4.5 kg), and his haemoglobin and ESR were 12.3 g/100 ml and 13 mm/h respectively. As is apparent, the diagnosis of collagen disease is tentative in this case.

METHODS

Regimen

Apart from case 1, all the patients were ambulatory. Forty-five were investigated for at least five days on a fixed dietary regimen, including 42 on a *standard diet*. This diet is wholefood diet containing approx. 800 mg Ca, 1,100 mg P 60–100 mEq Na, and 1 g protein per kg body weight in the 24 hours. Among patients with hyperparathyroidism *only* (cases 1–49) and among those *with non-parathyroid hypercalcaemia (cases 58–76)* 26 and 10 respectively were examined on a standard diet. The remaining 23 and 9 patients were either on a free diet or on diet differing essentially from the standard diet (cases 13 and 76). In the two groups on standard diet the mean content of calcium and phosphate was 831 ± 74 and 853 ± 97 mg Ca, and 1 150 ± 28 and 1,153 ± 37 mg P respectively in the 24 hours (mean ± S.D.) (Table I). The mean values comprise analysed as well as calculated diets (cf. Table I): the latter were corrected according to the following observations. During the period of the studies (1964–1969) 47 calcium and 26 phosphate balance studies were performed during which 142 and 80 dietary portions respectively were analysed. If the analytical results for calcium and phosphate are expressed in per cent of the values calculated according to the food tables, the mean values are +10.2 ± 11.8 % and +6.3 ± 6.0 % respectively (mean ± S.D.).

After analysing the patient material we studied the

effect of high calcium intake upon the TRCa% in six patients with hyperparathyroidism (*not* included in Table I). These patients were investigated first on *standard diet*, and thereafter on a *standard diet + approx. 1,500 mg calcium daily* for 2–4 days. The supplement of calcium consisted of Calcium Sandoz effervescent tablets, one tablet three times daily.

Collection of samples, analytical methods, and calculations

After two days on the diet, the blood sampling and urine collections were started. The blood was analyzed for TOCa, UFCa, Ca++, inorganic phosphorus (= serum phosphate = P), creatinine, and alkaline phosphatases, while the urine analyses comprised calcium, inorganic phosphorus, creatinine, and sodium. The blood sampling and urine collections, chemical analyses—apart from that of alkaline phosphatases (2)—and calculations of tubular reabsorption of calcium (TRCa%) and of the 24-hour phosphate excretion index (PEI) were performed as described elsewhere (18, 19). To allow a wider diagnostic use of the TRCa% method, we also calculated ~TRCa % substituting *UFCa by TOCa* in these calculations.

The number of TRCa% determinations ranged from 1–13 for each person. Table I gives chiefly the mean values for the TRCa% and the other parameters. Only when indicated by changes in factors affecting the TRCa% are the results presented separately.

Suppression tests were done on 19 patients. Initially we used prednisone, 10 mg three times daily. At present we use cortisone, 50 mg three times daily as recommended by Dent (4), but for a longer period, 12–20 days. Case 69 received 100 mg daily corresponding to about 150 mg/70 kg body weight. TOCa, and occasionally also UFCa and Ca++ were determined before and every 2nd–3rd day during the test. The TRCa% determinations were repeated in eight cases after more than ten days suppression.

RESULTS

Tubular Reabsorption of Calcium

Hyperparathyroidism, standard diet (n = 26)

TRCa% as well as ~TRCa % proved to be within a narrow range, decreasing slightly but significantly with increasing UFCa ($t = 3.28$, $p < 0.01$ and $t = 3.76$, $p < 0.001$) (Fig. 1). One patient exhibited a borderline value (case 44), cf. Discussion.

Hyperparathyroidism, free diet (n = 3)

Within this group TRCa% and ~TRCa % also showed a significant decrease with increasing UFCa ($t = 2.34$ $p < 0.05$ and $t = 2.15$ $p < 0.05$) (Fig.) Fig. 2 also shows that the TRCa values on a free diet are on the whole below those on a standard diet, although only four values were below the bottom limit of the standard diet group (cases 15, 26, 30 and 49). The difference between mean values of TRCa% for the two groups, 97.59 and 96.85% is statistically significant ($p < 0.05$), while this does not apply to the mean values for ~TRCa % 98.17 and 97.83% (Table III).

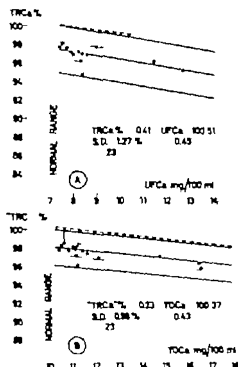


Fig. 2. The tubular reabsorption of calcium (TRCa%) in patients with *hyperparathyroidism* studied on *free diet* (n = 23). The hatched lines derive from Fig. 1. Concerning (A) and (B) see the legend of Fig. 1.

Hyperparathyroidism associated with other conditions influencing the calcium and phosphorus metabolism, standard or free diet (n = 8)

In this heterogeneous group the TRCa% and ~TRCa % proved to be within the hyperparathyroidism range in six out of eight patients (Fig. 3). The exceptions were case 51 (sarcoidosis) and case 53 (mastocytosis), cf. Discussion.

Hyperparathyroidism, high calcium diet (n = 6)

While UFCa did not alter significantly there was an increase in the renal calcium excretion and decrease in TRCa% in all six patients. On the

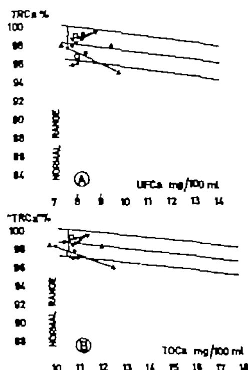


Fig. 3. The tubular reabsorption of calcium (TRCa%) in patients with *hyperparathyroidism combined with sarcoidosis*, nos. 50–51 (▲), *renal tubular acidosis*, no. 52 (▼), *mastocytosis, neurofibromatosis, chronic glomerulonephritis* and *rheumatoid arthritis during long-term treatment with prednisone calcium and vitamin D* nos. 53– (○). A case of *pseudohyperparathyroidism* is also presented, no. 57 (▭). The solid lines derive from Fig. 1, white and black symbols indicate the respective use of free and a standard diet. For further information, see Table I and the legend of Fig. 1.

average the calcium excretion increased by 90 mg/24 h ($p < 0.05$), while the TRCa% fell by 1.4% ($p < 0.05$) (Table II).

Table II. *Effect of high calcium intake upon the tubular reabsorption of calcium (TRCa%) in 6 patients with hyperparathyroidism*

		Standard diet	S.D + 1500[a]	Difference	p[b]
CCr	ml/min	75	71	−4	n.s.
UFCa	mg/100 ml	8.73	8.80	−0.07	n.s.
U-Ca	mg/24 h	289	379	+90	<0.05
TRCa	%	96.8	95.4	−1.4	<0.05

[a] Standard diet + 1500 mg Ca.
[b] p = probability determined by Wilcoxon rank test for pair differences; n.s. = non-significant.

Non-parathyroid hypercalcaemia standard or free diet (n = 19)

The main group comprises 18 patients (cases 58–75) all of whom exhibited a low TRCa% and "TRCa"% except for a single determination on free diet in case 64 (Fig. 4 A–B). The mean value for TRCa% is far below that of the hyperparathyroid group, 90.5% as compared with 97.3% ($p < 0.01$) (Table III). The last patient (case 76) showed the highest TRCa% value in the whole series, viz. 99.4% (Fig. 4 A–B), cf. Discussion.

Tubular reabsorption of calcium and glomerular filtration rate

Fig. 5 presents concurrent values of TRCa% and CCr in hyperparathyroidism (cases 1–57) and

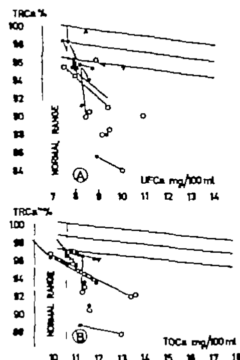


Fig. 4. The tubular reabsorption of calcium (TRCa%) in patients with non-parathyroid hypercalcaemia ($n = 19$). The following disorders are included: *vitamin D intoxication*, nos. 58–60, *sarcoidosis*, nos. 61–69, *collagen disease* nos. 70–71 and *malignant lymphomas*, nos. 72–73 all of them being presented by the same symbol (○) added to these are single cases of *myelomatosis*, no. 74 (▽), *idiopathic intestinal hyperabsorption hypercalcaemia*, no. 75 (▭) and *hypokalaemic alkalosis due to vomiting*, no. 76 (△). White and black symbols indicate the respective use of a free and standard diet. For further explanation, see legend to Fig. 3.

Table III. *Comparison of groups of patients with hyperparathyroidism tested on standard diet and on free diet and between patients with hyperparathyroidism and patients with non-parathyroid hypercalcaemia*

	Hyperparathyroidism			Nonpt. h.*	Significance of differences	
Diet	Standard $n = 26$	Free $n = 23$	Combined $n = 49$	Combined $n = 18$	p	p
Group no.	1	2	3	4	1 2	3 4
TOCa (mg/100 ml)	12.35	11.85	12.12	12.23	>0.05	>0.05
UFCa (mg/100 ml)	9.30	8.85	9.09	9.39	>0.05	>0.05
UFCa (% of TOCa)	—	—	74.9 ±2.4	76.6 ±3.9	—	>0.05
P (mg/100 ml)	2.76	2.79	2.77	3.48	>0.05	<0.01
CCr (ml/min)	92.1	81.4	87.1	49.4	>0.05	<0.01
Urinary Ca (mg/24 h)	287	316	301	537	>0.05	<0.01
TRCa %	97.39 ±0.84	96.85 ±1.27	97.24	90.49	<0.05	<0.01
†TRCa %	98.17 ±0.55	97.63 ±0.96	97.92	92.74	>0.05	<0.01

* Non-parathyroid hypercalcaemia (cases no. 58–75).
† Wilcoxon rank test for two samples.

in non-parathyroid hypercalcaemia (cases 58–75). As far as the latter group is concerned, it is evident that the TRCa% decreased with decreasing CCr while the hyperparathyroid group showed only a modest tendency in this direction.

24-Hour Urinary Calcium Excretion

Hyperparathyroidism standard free diet ($n = 49$)

In this group it is worth noting that the mean value was no higher than 301 mg/24 h (Table III)—in spite of TOCa, UFCa and CCr values of 12.1 mg/100 ml 9.09 mg/100 ml, and 87 ml/min, respectively The calcium excretion was *normal* in 21 patients (43%) *only* eight of whom had a CCr value of less than 75 ml/min (Table IV).

Non-parathyroid hypercalcaemia, standard or free diet ($n = 19$)

In spite of approximately the same TOCa and UFCa and an appreciably lower CCr 49 ml/min ($p < 0.01$), the main group is characterized by an *invariable and pronounced hypercalciuria* whose mean value, 537 mg/24 h, by f r exceeded that of the hyperparathyroid group ($p < 0.01$) (Table III) Normocalciuria was observed only in the patient with an excessively high TRCa% (case 76) (Table IV).

Serum Phosphate

Among 56 patients with *hyperparathyroidism* 10 of whom had a CCr of less than 50 ml/min, only 1 (21%) had hypophosphataemia (Table V). Conversely only four serum phosphate levels

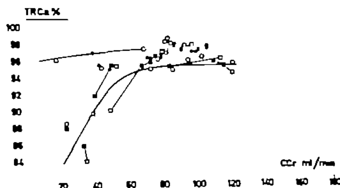


Fig 5 The tubular reabsorption of calcium (TRCa%) related to the 24-hour clearance of creatinine (CCr) in hyperparathyroidism, nos. 1 57 ( ) and in non-parathyroid hypercalcaemia, nos. 58–75 ( ) patient no 76 was omitted. White and black symbols indicate the respective use of free and standard diet. The solid lines are approximations indicating the average relation between TRCa% and CCr in hyperparathyroidism (the upper line) and in non-parathyroid hypercalcaemia (the lower line)

Table IV *Renal excretion of calcium in hyperparathyroidism (n = 57) and in non-parathyroid hypercalcaemia (n = 19)*

	CCr (ml/min)	Renal calcium excretion: Normal	Renal calcium excretion: High[a]	Total
Hyperparathyroidism (nos. 1–49)	>75	13	22	35
	<75	8	6	14
Total		21	28	49
Hyperparathyroidism (nos. 50–57)	>75	1	0	1
	<75	5	2	7
Total		6	2	8
Hyperparathyroidism (nos. 1–57)	>75	14	22	36
	<75	13	8	21
Total		27	30	57
Non-parathyroid hypercalcaemia (nos. 58–76)	>75	1	3	4
	<75	0	15	15
Total		1	18	19

[a] Females, >250 mg/24 h. Males, >300 mg/24 h (10).
[b] Hyperparathyroidism associated with other conditions influencing calcium and phosphorus metabolism.

were within the upper half of the normal range (3.6–4.6 mg/100 ml) and only one of these patients had a normal CCr viz. the one with hyperparathyroidism and neurofibromatosis (case 53).

None of the patients with *non-parathyroid hypercalcaemia* had hypophosphataemia, but the serum phosphate concentration was in the lower half of the normal range in half the patients.

The 24-Hour Phosphate Excretion Index

At CCr < 50 ml/min the PEI was increased, *irrespective of the cause of hypercalcaemia* (n = 22, Table VI). At a CCr ≥ 50 ml/min the PEI was increased in 30 out of 46 within the *hyperparathyroid* group (65%) and in four out of seven within the *non-parathyroid* group.

Serum Alkaline Phosphatases

The concentration of alkaline phosphatases was elevated in 45% of the patients with *hyperparathyroidism* and in 33% of those with *non-parathyroid hypercalcaemia* (Table VII).

Suppression Tests

Effect on serum calcium

Cortisone had no definite effect upon the TOCa in three patients with *hyperparathyroidism* (Fig. 6), one of whom had sarcoidosis and one, possibly several, parathyroid adenomas (case 50).

Table V *Concentration of serum phosphate assessed on the basis of the 24-hour clearance of creatinine (CCr) in hyperparathyroidism (n = 56) and in non-parathyroid hypercalcaemia (n = 19)*

	CCr (ml/min)	Serum phosphate[a] (mg/100 ml): <2.5	2.5–2.9	3.0–3.5	3.6–4.6	Total
Hyperparathyroidism (nos. 1–49)	≥50	10	20	11	0	41
	<50	2	2	2	1	7
Total		12	22	13	1	48[b]
Hyperparathyroidism (nos. 50–57)	≥50	0	0	4	1	5
	<50	0	1	0	2	3
Total		0	1	4	3	8
Hyperparathyroidism (nos. 1–57)	≥50	10	20	15	1	46
	<50	2	3	2	3	10
Total		12	23	17	4	56
Non-parathyroid hypercalcaemia (nos. 58–76)	≥50	0	2	2	3	7
	<50	0	1	5	6	12
Total		0	3	7	9	19

[a] Normal range 2.5–4.6 mg/100 ml.
[b] Case no. 32 not examined.
Hyperparathyroidism associated with other conditions influencing calcium and phosphorus metabolism.

In case 51 with sarcoidosis and parathyroid hyperplasia, the hypercalcaemia decreased during three suppression tests (Fig 6) while the UFCa and Ca++ remained clearly elevated during a 20-day test (Table VIII)

Three of the patients with *non-parathyroid hypercalcaemia* experienced a spontaneous and complete remission during or immediately after the TRCa% study (cases 58, 65 and 70) Suppression tests were performed on 15 of the remaining 16 patients (Fig. 7). With two exceptions (cases 71 and 75) the TOCa fell definitively in the course of less than ten days. In case 71 it was still borderline on the 13th and 15th day while UFCa and Ca++ were normal (Table VIII). In *idiopathic intestinal hyperabsorption hypercalcaemia* (case 75) the hyperabsorption was cortisone-resistant (12) and therefore the TOCa, UFCa, and Ca++ remained unchanged. The observations on UFCa and Ca++ are still too few to detect when the normalization usually sets in (T ble VIII)

Table VI *The 24-hour phosphate excretion index assessed on the basis f the 24-hour clearance of creatinine (CCr) in hyperparathyroidism (n = 56) and in non-parathyroid hypercalcaemia (n = 19)*

	CCr (ml/min)	24-h phosphate excretion index Normal	High[a]	Total
Hyperparathyroidism (nos. 1–49)	> 50 < 50	15 0	26 7	41 7
Total		15	33	48
Hyperparathyroidism (nos. 50–57)	> 50 < 50	1 0	4 3	5 3
Total		1	7	8
Hyperparathyroidism (nos. 1 57)	> 50 < 50	16 0	30 10	46 10
Total		16	40	56
Non-parathyroid hypercalcaemia (nos. 58–76)	> 50 < 50	3 0	4 12	7 12
Total		3	16	19

Above +0 09 (18).
Case no. 32 not examined.
Hyperparathyroidism associated with other conditions influencing calcium and phosphorus metabolism.

Table VII *Serum alkaline phosphatases in hyperparathyroidism (n = 55) and in non-parathyroid hypercalcaemia (n = 18)*

	Alkaline phosphatases Normal	High[a]	Total
Hyperparathyroidism (nos. 1–49)	24	23	4
Hyperparathyroidism (nos. 50–57)	6	2	8
Total	30	25	55
Non-parathyroid hypercalcaemia (nos. 58–76)	12	6	18

Above 38 normal range 13–38 U (= μmol min) 1
[a] Cases nos. 22, 32 and 62 were not examined
Hyperparathyroidism associated with other conditions influencing calcium and phosphorus metabolism

Effect on TRCa%

The TRCa% did not alter notably in cortisone-resistant hypercalcaemia (cases 50 and 75 d) In the group of patients with cortisone-*sensitive* non-parathyroid hypercalcaemia (cases 63 66–67 69 and 71) the TRCa% rose towards a level of 94 7–96.2% i.e. lower than normal (Fig. 8). In contradistinction, the patient with sarcoidosis and parathyroid hyperplasia (case 51) who was

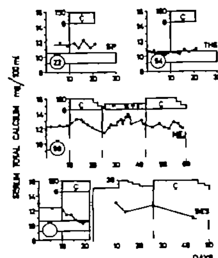


Fig 6. Cortisone (C) and prednisone (P) suppression tests in *hyperparathyroidism* (nos. 22 and 54) and in *hyperparathyroidism associated ith sarcoidosis* (nos. 50 and 51) In patient no. 51 who had an increased intestinal absorption of calcium, serum total calcium decreased on three occasions.

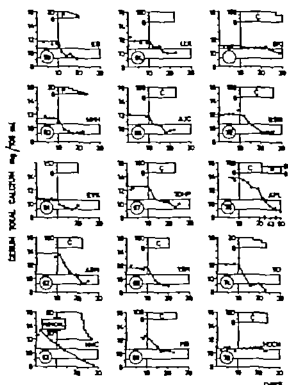


Fig. 7. Cortisone (C) and prednisone (P) suppression tests in non-parathyroid hypercalcaemia: *vitamin D intoxication* (nos. 59–60), *sarcoidosis* (nos. 61–69), *collagen disease* (no. 71), *malignant lymphomas* (nos. 72–73), *myelomatosis* (no. 74), and *idiopathic intestinal hyperabsorption hypercalcaemia* (no. 75). Serum total calcium decreased in all patients except no. 73.

also cortisone *sensitive*, responded by a TRCa% increase to 98.0%, a value in the middle of the hyperparathyroidism range (cf. Discussion).

DISCUSSION

TRCa% in Hyperparathyroidism

Effect of parathyroid hormone on TRCa%

The effect of the parathyroid hormone has previously been described in detail (18), but a few features deserve emphasis. If the filtered load of calcium in a normal individual is increased, e.g. by i.v. infusion of calcium salts, tubular reabsorption will increase. However, the increase of reabsorption is not fully proportional to that of filtration. Accordingly, tubular reabsorption will exhibit a decreasing tendency *when expressed in per cent of the filtered load (TRCa%)*. If the calcium infusion is repeated after pre-treatment with parathyroid extract, the same pattern is found, but *at a given concentration of UFCa* the TRCa% will be higher than without pretreatment (1, 13).

Injection of parathyroid hormone into a parathyroidectomized person or experimental animal is followed by an *initial* fall in renal calcium excretion which, however, again increases up to or beyond the initial level (3, 7, 15). If the parathyroid hormone exerted *only* this renal effect, the UFCa would increase only *until* the calcium excretion had reached the initial level, since then the balance between intake and output would be re-established. A higher dose of para-

Table VIII. *Effect of cortisone or prednisone upon serum total calcium (TOCa), ultrafiltrable calcium (UFCa) and ionized calcium (Ca^{++}) in 14 patients with hypercalcaemia*

Pat. no.	Type of steroid[a]	Dose of steroid (mg/24 h)	Test day	TOCa (mg/100 ml)	UFCa (mg/100 ml)	Ca^{++} (mg/100 ml)
Primary hyperparathyroidism						
22	C	150	12	11.7	8.85	7.95
Hyperparathyroidism and sarcoidosis						
50	P	15	18	12.9	9.85	8.95
51	C[b]	150	10	10.[illegible]	8.10	7.60
—	—	—	12	10.5	8.45	7.40
51	C[c]	150	18	11.0	8.30	7.40
—	—	—	21	10.4	7.95	7.15
Hyperparathyroidism and neurofibromatosis						
54	C	150	12	11.0	—	7.50
Ordinary hypercalcaemic sarcoidosis						
61	P	10	34	10.0	7.55	6.60
63	P	75	22[e]	8.9	6.95	6.30
64	C	150	14	10.1	7.70	6.90
66	C	150	11	9.7	7.55	6.80
—	—	—	13	9.8	7.40	6.55
67	C	150	11	10.7	7.70	6.80
—	—	—	13	10.0	7.40	6.35
69	C	100[f]	12	10.3	7.90	6.75
—	—	—	14	10.1	7.70	7.15
Remittent hypercalcaemia (collagen disease?)						
71	C	150	13	10.9	7.40	6.30
—	—	—	15	10.5	7.30	6.10
—	—	—	20	10.0	7.40	—
Malignant lymphomas						
72	C	150	17	9.9	7.75	6.60
Idiopathic intestinal hyperabsorption hypercalcaemia						
75	C	150	14	10.8	7.95	7.10

[a] C – cortisone, P – prednisone.
[b] and [c] Cortisone tests I and II.
[d] During maintenance therapy.
[e] Five days after the termination of intensive prednisone therapy.
[f] Approximately 150 mg/70 kg body weight.

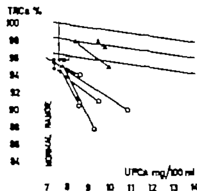


Fig. 8. The tubular reabsorption of calcium (TRCa %) *before* (blue symbols) and *after* more than ten days of cortisone or prednisone administration (black symbols). The following cases are included: *sarcoidosis*, nos. 63, 66–67 69, and *collagen disease* no 71 (○), *idiopathic intestinal hyperabsorption hypercalcaemia*, no. 75 (□), and *hyperparathyroidism combined with sarcoidosis*, nos. 50–51 (△). The rise from low value (no. 51) to value situated in the middle of the range of hyper parathyroidism is of special interest (see text).

thyroid hormone would afford a higher UFCa, but the same calcium excretion in other words, the TRCa % would *rise* However the effect of the parathyroid hormone upon bone resorption and upon intestinal absorption of calcium increases the UFCa *beyond* what is occasioned by the renal effect; *therefore* hyperparathyroidism is frequently associated with an increased renal excretion of calcium. Since the share of extrarenal factors in hypercalcaemia must be assumed to increase with the severity of the hyperparathyroidism, it must be expected that—*ceteris paribus* —the *TRCa % will decrease with increasing UFCa*, and indeed this was what we found (Figs. 1 A and 2 A).

Effect of non-parathyroid factors upon TRCa % in hyperparathyroidism

Factors acting upon the bone resorption or the intestinal absorption of calcium must be able to affect the UFCa and thus also the TRCa % *Increased bone resorption*, e.g. in immobilization or osteolytic metastases, *reduces* the TRCa % and this applies also to increased intestinal absorption of calcium due to excessive calcium intake or excessive absorptive capacity (case 51). Since, for practical reasons, we calculate the TRCa % on the basis of the *24-hour* excretion of calcium and of the UFCa determined *in the morning in the fasting state* it is important to point out that a *transient* increase in UFCa after excessive calcium intake gives rise to a fall in TRCa % *apparently* without any increase in UFCa (Table II) *Increased deposition of calcium* e.g. due to increased intake of phosphate, and a *reduced absorption of calcium* as a result of malabsorption (case 57) or dietetic calcium restriction (case 49), may of course *increase* the TRCa %

Factors exerting a *direct renal effect upon tubular reabsorption*, such as changes in sodium reabsorption, diuretics, and changes in the acid base status (case 5., Fig. 3), may modify the effect of the parathyroid hormone upon the TRCa %

Diagnostic applicability of TRCa % determinations in hyperparathyroidism

Comparison of our TRCa determinations in the 57 patients with our previous hyperparathyroidism range (18)—which is practically identical with that of the group on standard diet (Fig. 1 A)—shows that the standard diet group included one borderline value, while the group on free diet included four and the combined group two low values (Fig. 1 A–3 A). Thus, the TRCa % determination confirmed the diagnosis of hyper parathyroidism in 50 out of 57 patients (88 %).

Diagnostic failures

Four failures occurred on free diet (cases 15 26 30 and 49). Thus, an *excessive intake of calcium* (cf Table II) is a reasonable explanation. The last-mentioned patient remained hypercalciuric after parathyroidectomy (case 49 *c*) in spite of a sustained normalization of the Ca^{++} (19). Therefore his condition was originally interpreted as idiopathic hypercalciuria co-existing with hyperparathyroidism (18). This possibility still exists, but the response to dietary calcium restriction (case 49 *b* and *d*) does indicate excessive calcium intake as the most probable explanation. Considering T ble II, these four failures definitely suggest *the use of a standard diet* Presumably it is immaterial whether our *standard diet or e.g.*, a low-calcium diet is used, but if the latter is selected, new hyperparathyroidism range must be established.

The patient with sarcoidosis and parathyroid hyperplasia had increased intestinal absorption

of calcium (11) and a *reduced* TRCa% (case 51 a, Fig. 3). A high TmG/GFR and an incomplete response to cortisone (Table VIII) led to the removal of thre hyperplastic parathyroid glands (20), and three months later follow-up showed entirely normal conditions (case 51 c Fig. 3) The reduced TRCa% which represents an indubitable diagnostic failure, was most probably due to the fact that in this patient the hyperparathyroidism co-existed with *hyperabsorptio of calcium due to sarcoidosis*. This view is supported by the effect of the cortisone test upon the TRCa% when the sarcoidosis component was suppressed, the TRCa% rose to the level conditioned by the hyperparathyroidism per se (case 51 b Fig. 8).

The last two failures concern patients in whom creatinine analyses were performed simultaneously Compared with previous investigations on a free diet (cases 44 a and 53 a) the simultaneous investigation revealed an appreciable fall in CCr and TRCa% (cases 44 b and 53 b Figs. 1 and 3) In both an increase of the serum creatinine concentration of 0.2–0 4 mg/100 ml was recorded, a finding which could not be reproduced later *An error in the analysis of creatinine* is the most probable explanation of the strikingly low TRCa% values during the second investiga Increased bone resorption due to masto- may have contributed to the pre-existence of a relatively low TRCa% in case 53 (Fig. 3).

TRCa% in Pseudohyperparathyroidism

In case 57 who had an oat-cell carcinoma of the pancreas *without* skeletal metastases, but complicated by severe steatorrhoea, the TRCa% was very high, as might be expected, 99.0% (Fig. 3) The differentiation between hyperparathyroidism and pseudohyperparathyroidism must still be based upon non-endocrinological methods.

TRCa% in Non-parathyroid Hypercalcaemia

TRCa% in overflow hypercalcaemia

This term is taken to comprise the non-parathyroid disorders which give rise to hypercalcaemia either by increasing bone resorption or intestinal absorption of calcium or by making metastatic calcifications undergo resorption in phases of remission (11) (cases 58–75). The hypercalcaemia inhibits the secretion of parathyroid hormone (14) resulting in a reduction of tubular reabsorption of calcium (1 13 16, 17).

TRCa% in retention hypercalcaemia

The term *retention hypercalcaemia* indicates hypercalcaemia due exclusively to a direct augmenting action of a non-parathyroid factor upon the tubular reabsorption of calcium. Such an effect is exerted by *metabolic alkalosis* and possibly also by *administration of thiazides*. This type of hypercalcaemia must of course inhibit the secretion of parathyroid hormone also, but the inhibition cannot manifest itself in the tubular reabsorption of calcium Retention hypercalcaemia is exemplified in case 76, whose TRCa% was extremely high, 99 4% due to vomiting-conditioned hypokalaemic alkalosis. Overdosage of sodium bicarbonate to the patient with renal tubular acidosis and hyperparathyroidism had a similar effect upon TRCa% and thereby also upon UFCa (case 52, Fig. 3)

Diagnostic applicability of TRCa% determinations in non parathyroid hypercalcaemia

The diseases responsible for hypercalcaemia and the precautions we took to rule out simultaneous hyperparathyroidism were mentioned under Material. Fig. 4 A shows that the TRCa% *at a given concentration of UFCa* was lower in this group than in primary hyperparathyroidism. The exceptions are the patient with retention hypercalcaemia, and one of the patients with sarcoidosis (case 64 a). Thus, a reduced TRCa% could confirm the diagnosis of non-parathyroid hypercalcaemia in 17 out of 19 patients (89%).

The explanation of the great scatter of the TRCa% values in this group (Fig. 4 A) is, as already pointed out (18), that the reduction of the TRCa% occasioned by hypercalcaemia depends to a great extent upon the existing reduction of the CCr (Fig. 5) This relationship between TRCa% and CCr evident at a CCr below 60 ml/min, is observed only to a minor extent in hyperparathyroidism. The reason for the decreasing TRCa% is presumably an increasing osmotic load per nephron, a factor which parathyroid hormone appears to be able to counteract quite effectively From a practical point of view this means *that the diagnostic accuracy of*

the TRCa% determinations increases with de creasing CCr at least within the CCr range in question (13–177 ml/min).

Diagnostic failure Case 64 had one slightly elevated TRCa% value on a *free* diet, but eight determinations on a standard diet showed a low level (cases 64 *a* and *b*)

True, the very high TRCa% in case 76 represents a diagnostic failure, but this may easily be recognized by the following precautions: the patients should be questioned concerning vomiting and consumption of alkali, and diuretics should be withdrawn at least one week before diagnostic TRCa% determinations. As a matter of course, determination of the TRCa% is not applicable for differentiating between hyperparathyroidism and the milk-alkali syndrome.

Differentiation between Hyperparathyroidism and Non-parathyroid Hypercalcaemia by Other Methods

Determinations of *alkaline phosphatases in the serum* and the *phosphate excretion index* are of no use (Tables V–VI Results), while analyses of *serum phosphat* and determinations of the *24-hour renal xcretion of calcium* are applicable within certain limits. Their range of application may be summarized as follows. 1) Manifest hypophosphataemia indicates hyperparathyroidism, but it occurs in only 21% (Table V). 2) Serum phosphate concentrations in the *upper half* of the normal range afford an important argument against hyperparathyroidism—when the CCr exceeds 50 ml/min (Table V) and acromegaly may be excluded (21). 3) A *normal* calcium excretion was found in 47% of the patients with hyperparathyroidism (Table IV) and greatly *supports* this diagnosis—if overdosage of bicarbonate, thiazide medication, and vomiting alkalosis can be ruled out.

The *cortisone test* and the *TmG/GFR* were performed only in selected cases and therefore cannot be systematically assessed. The *cortisone test* has been negative in 45 out of 47 patients with hyperparathyroidism (96%) when patients with generalized osteitis fibrosa could be excluded (5). At the same time, it has been reported to be 100% positive in an unstated number of patients with hypercalcaemic sarcoidosis, vitamin D intoxication, and idiopathic hypercalcaemia of infancy while the effect was said to be variable in patients with cancer (5) We recorded two failures among 19 patients (cases 51 and 75). Halver found an elevated TmG/GFR in 29 out of 37 patients with hyperparathyroidism (78%) but the value of this test is greatly restricted by the fact that patients with water clear cell hyperplasia have a low TmG GFR, just like patients with non-parathyroid hypercalcaemia (9).

Practical Use of the TRCa% D terminations

The optimal demands on a diagnostic test are that it should be dependable, harmless, and easy In respect to the dependability we consider the requirement to be fulfilled. Under the present conditions the diagnosis we could make by other means has been confirmed in 88% of the patients, and we have reason to assume that the systematic use of a fixed calcium intake will raise the diagnostic accuracy somewhat beyond the 90% limit. The advantages of the TRCa% determination over the cortisone test are *that* it may be performed regardless of whether the patient has peptic ulcer hypertension, etc *that* it is quicker *that* it does not induce hypocorticism prior to possible parathyroidectomy and *that* it may be performed without interfering with calcium balance studies.

The calculation of TRCa% presupposes determination of the UFCa, and this has prevented its more widespread use. However as also pointed out previously (16), it has been found that *calculation of the "TRCa % on th basis of the TOCa instead of the UFCa ffords the same qualitative separation of the patient groups as TRC %* (Figs. 1 B–4 B) The explanation is that for this purpose UFCa makes up sufficiently constant fraction of TOCa, viz. 74.9 ± 2.4% in hyperparathyroidism and 76.6 ± 3.9% (mean ± S.D.) in non-parathyroid hypercalcaemia ($p > 0.05$ Table III).

The detection of hypercalcaemia is presupposition for the diagnostic use of "TRCa % as well as TRCa%. The determinations which are situated, in Figs. 1–2, *within* the respective normal ranges of TOCa and UFCa are from patients whose hypercalcaemia had been established by determination of Ca^{++} This minority will of course escape assessment if the hypercalcaemia

is defined as an elevated concentration of TOCa or UFCa. We realize that from the point of view of renal physiology "TRCa % is a coarse distortion, but we feel justified in pointing out that our data afford an *empiric* basis for the *diagnostic* use of "TRCa % determinations.

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PROGNOSTIC SIGNIFICANCE OF ECG-CHANGES IN SURVIVORS OF MYOCARDIAL INFARCTION

Five year Follow-up Study

Paul Leren, Ingvar Hjermann and Jan Bjørnson

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Abstract. Electrocardiograms recorded 1–2 years after first myocardial infarction in 412 males have been classified according to the Minnesota Code, and ECG groups of possible prognostic significance were established. One reference group with normal or fairly normal ECGs; three other groups: (A) with major Q-changes, (B), with Q- and T-changes, and (C), mixed group with hypertrophy conduction defects and arrhythmias. The 5-year incidence of myocardial reinfarction and of acquired angina pectoris was uninfluenced by the ECG pattern at the start of the study. The incidence of sudden death and total CHD death was lower when the ECG at the start of the study was normal or fairly normal.

The ECG has played an important role in epidemiologic studies of coronary heart disease. The accuracy of ECG screening of large population groups was increased by the introduction of the standardized ECG classification systems of the Minnesota Code (2). This system has proved to be useful in the study of large groups of presumedly healthy people (4, 6, 9). Whether the ECG classification system is useful also in the evaluation of prognosis in postmyocardial infarction patients has, to our knowledge, not yet been studied.

The present study is an attempt to settle whether certain ECG changes in survivors of a first myocardial infarction are related to recurrent coronary events.

MATERIAL AND METHODS

The study deals with the ECGs of 412 survivors of first myocardial infarction, described by one of us (P. L.) in the Oslo diet heart study (5). Mean age of the patients at entry was 56 (30–64). At the start of the study 12-lead resting ECG was recorded, including the standard leads I, II and III, and the unipolar leads VR, VL, VF and V_{1-6}. This first ECG recorded 1–2 years, on average 20 months, after the primary infarct, has been coded according to the revised Minnesota Code (7). The coding was performed by two of us (J. B. and I. H.), independently of one another and in ignorance of coronary events during the follow-up period. The results of each individual coding were compared and differences discussed. Of the 412 ECGs common coding was arrived at in all but 16, in which third reader (P. L.) was summoned to settle the question. Finally the ECGs were divided into four groups according to the following criteria.

Group "0" ("normal" or reference group), defined in Table I. The miscellaneous items in Table I include "normal" ST-elevation (code 9–2) in lead V when no other codable items were present, tall P-waves (code 9–3), counterclockwise and clockwise rotation (code 9–4–1, 9–4–2) and tall T-waves (code 9–5).

Group A. Pronounced Q-changes (code 1–1 and 1–2) plus any other *additional* ECG changes.

Group B. Group A changes plus low-grade Q-changes (code 1–3) or "coronary" T (code 5–1, 5–2) without Q-changes.

Group C (mixed group). Ventricular hypertrophy (code 3), pronounced ST-changes (code 4–1, 4–2 and 5–1, 5–2), A–V conduction defects (code 6), pronounced ventricular conduction defects (code 7–1, 7–2, 7–4, 7–6), low voltage (code 9–1), *arrhythmias* (code 8), *except* sinus tachycardias and sinus bradycardias.

It is obvious that some of the ECGs were allocated to more than one group, e.g. Q-changes (code 1–1, 1–2) may be found in group A as well as in group B, and T-changes without Q-changes (code 5–1, 5–2) in group B and group C. However, the reference group "0" is strictly separated from the other groups.

In conclusion the groups A, B and C represent major pathological ECG patterns reflecting myocardial injury (groups A and B), and hypertrophy, conduction defects and arrhythmias (group C). The reference group, however, includes no ECG with definite signs of myocardial damage.

Table I. *ECG-items in "normal" (reference) group*

Items	Minnesota code
No codable items	0
Axis deviations	2
Minor ST–T-changes	4–3
	4–4
	4–2 when combined with 5–3
Minor T-changes	5–3
Minor ventr. conduction defects (low-grade RBBB)	7–3
	7 5
Sinus tachy/bradycardia	8–7
	8–8
Miscellaneous items	9–2 (partly see text)
	9–3
	9–4–1
	9–4–2
	9 5

Close clinical and laboratory follow-up examinations were undertaken of each of the 412 patients for exactly five years or until death, and the incidence of recurrent coronary events (CHD relapses), as defined below has been related to the four "prognostic ECG groups. In each comparison only one CHD relapse has been counted in each patient. For the combined incidence of myocardial reinfarction and acquired angina pectoris, reinfarction has been given priority over new angina.

It should be remembered that, whereas all patients ran risk of reinfarction r sudden death, new angina could only arise in the 153 patients who at the start of study were free from this condition. Tests of significance have been made by simple X tests.

Definitions / CHD relapses (described in detail in ref. 5)

1. Myocardial reinfarction
   (*a*) Fatal and non-fatal myocardial reinfarction according to preestablished criteria,
   (*b*) Fatal episodes with chest pains preceding death No clinical or laboratory diagnosis established (type II sudden death in ref. 5).

Table II. *ECG groups of possible prognostic significance*

ECG groups	Code	No
A (Q)	1 1 1 2	196
B (Q+T)	1 1 1 2, 1 3, 5–1 5–2	280
C (min)	3, 4–1 4–2, 5–1 5–2, 6, 7 8, 9–1 9–2	61
0 ("normal")	See Table I	107

2. Acquired angina pectoris based on characteristic symptoms
3. Sudden death
   (*a*) Instantaneous death (type I in ref. 5).
   (*b*) Unwitnessed death (type III in ref. 5).

RESULTS

The reading of the 412 ECGs resulted in 67 (16.3 %) without codable items according to the Minnesota Code. The distribution of the ECGs in the four prognostic groups is presented in Table II.

It will be seen that the normal" or "0" group (reference group) according to the above mentioned criteria, also includes some minor insignificant ECG changes and in total 107 (25.9 %) of all the ECGs.

Fig. 1 presents the incidence of myocardial reinfarction and new angina pectoris in relation to the four prognostic" groups. It appears that the ECG does not influence the 5-year combined incidence of myocardial reinfarction and acquired angina pectoris. However the incidence of sudden death (Fig. 2) is significantly lower when the ECG at the start of the observation period is normal or fairly normal. After excluding cases with nonfatal CHD relapses, the incidence of sudden death still tends to be lower when the ECG is normal. However this difference is not statistically significant.



Fig. 1 Five-year incidence of myocardial reinfarction and acquired angina pectoris in relation to ECG changes.

Also the total incidence of CHD deaths (fatal myocardial reinfarction and sudden death) is significantly lower when the initial ECG is normal (Fig. 3).

DISCUSSION

Prospective epidemiologic studies of large population groups have shown that certain ECG abnormalities reflect an increased risk of developing clinical coronary heart disease (1 3 4 6, 8). On the other hand, the prognostic significance of a normal ECG prior to overt coronary disease is much limited. The "healthy person coming for his annual coronary check presenting a normal resting ECG and who on the next day has a heart attack, is well known. After all, such disappointing experience seems logical. It should be remembered that the resting ECG at best mirrors the state of the myocardium, telling nothing about the state of the coronary arteries.

The present study compares certain ECG changes in postmyocardial infarction patients with later fatal and non-fatal coronary events. When evaluating the results of this study it should be borne in mind that the ECGs were recorded at least one year after the primary infarct. Thus the results do not apply to the immediate prognosis after a myocardial infarction.

It is demonstrated that, in these postmyocardial infarction patients, the combined incidence of myocardial reinfarction and of new angina pectoris is uninfluenced by the pattern of ECG changes, and by whether the ECG is normal or not. This is not so with regard to sudden death. The incidence of sudden death, as well as the total CHD mortality is significantly lower when the ECG is normal than when the ECG is pathologically changed at the start of the observation period.

These observations suggest that the ECG in survivors of a myocardial infarction is bad predictor with regard to developing a new myocardial infarction. However with a normal ECG the risk of sudden death seems to be lower than when the ECG is pathologically changed.

A possible explanation of this observation might be that, whereas the risk of a new infarct or acquiring angina is related to the degree of coronary atherosclerosis of which the resting ECG tells nothing, the death risk is more related to the state of the myocardium, which more likely is reflected in the ECG

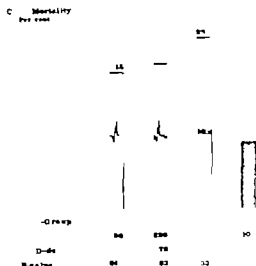


Fig. 3. Five-year incidence of total CHD mortality (fatal reinfarction + sudden death) in relation to ECG changes

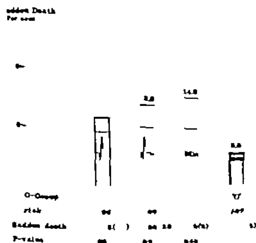


Fig. 2. Five-year incidence of sudden death in relation to ECG changes. In parentheses sudden death cases as first CHD relapse.

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FUNCTIONAL CHECKING OF IMPLANTED CARDIAC PACEMAKERS

Methods, Values Indicative of Impending Pacemaker Failure and Results of Testing of Fixed Rate Ventricular Synchronous and Atrial Synchronous Pacemakers

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Abstract. The present work introduces the results of oscilloscopic analysis of pacemaker impulse curves for pacemakers and electrodes of Elema-Schönander type. The measuring method is described, and few methodological studies are introduced. Comments are made on the checking of synchronous pacemakers. Values indicating impending pacemaker faults for the types of pacemakers used are presented. A material of 56 exchanged pacemakers is accounted for in detail. Seventeen of these pacemakers have been exchanged as a matter of routine after 18 months of service. Eleven of these 17 were intact, both as regards analysis before the exchange and as regards the checking of the pacemaker itself after explantation. Four pacemakers had to be exchanged owing to clinical symptoms of defective stimulation. With the present values indicative of exchange one of these would have been exchanged in good time before the onset of the symptoms. In 31 cases the exchange has taken place because of a defective impulse curve, and none of these pacemakers had given any clinical symptoms of stimulation fault. Only four of these 31 would have been revealed as defective if only pulse counting and ECG had been the basis of pacemaker checking. The reasons for premature failures in the pacemakers used are discussed. Different methods of pacemaker checking and different forms of the oscilloscopical analysis are brought up. The advantages of checking method of the type used is pointed out and the article is concluded with some views on prospects regarding pacemaker checking.

Detailed studies on the electrical performance of a pacemaker system were previously made at the time of repair of various types of stimulation defects (16). The checking of pacemaker-treated patients primarily consisted of a clinical examination and an electrocardiogram. However not uncommonly acute symptoms arose as a result of defects in stimulation (27). It was subsequently found that defects in the pacemaker system do not usually arise suddenly (30). In other words, detection of a number of kinds of defects should be possible before they become so serious that the pacemaker ceases to stimulate the heart.

A prerequisite, however, is that the electrical performance of the pacemaker system can be checked continuously so that data can be obtained which permit replacement of a defective part in the system before the patient feels the defect as a stimulation failure.

Of the various suggested checking methods it would appear that the oscilloscopic analysis of the pacemaker impulse curve has been most used. Despite the fact that a number of authors (10, 17, 22, 25, 29) have described the use of this method, only very few reports have dealt with a uniformly treated and followed-up pacemaker series. There would appear to be no such report for the pacemaker system used by us. We therefore consider it justified to report our experience with this method and to discuss its clinical value. At the same time we present some aspects on methods of measurement and values indicative of impending pacemaker failure for the pacemaker used in this study.

MATERIAL AND METHODS

Since January 1966 a total of 110 pacemaker patients have been treated at the Department of Medicine, Falun Hospital. Since September 1966 the performance of the pacemakers has been evaluated by oscilloscopic analysis. The material to be described consists of 56 pacemakers which were replaced up to the end of September 1969. The reason for replacement in each case has been stimulation defect caused by a fault in the pacemaker stimu-

Table I. *Distribution of the exchanged pacemakers by types*

Type of impulse generator	No
Fixed rate EM 139	17
Fixed rate EM 142	31
Ventricular synchronized, EM 143	7
Atrial synchronized, EM 141	1
Total	56

urement values indicative of impending failure, or routine replacement after 18 months service. Pacemakers still functioning satisfactorily but replaced before 18 months for reasons other than those mentioned above (e.g. in the change-over from fixed rate to synchronous pacing), as well as pacemakers not checked over an 18-month period, have not been included in the material. The various types of pacemakers included are shown in Table I. The majority (48) are fixed rate, but seven are ventricular synchronous, and one atrial synchronous. These pacemakers were used in 38 patients, and the methodological studies were carried out on them.

Surgical Technique

I practically all cases the pacemaker technique consisted of endocardial pacing with monopolar electrode inserted via the external or internal jugular vein. The pacemakers were implanted in the subcutaneous fat of the abdomen below the left costal arch, with the indifferent electrode placed close to the pacemaker (11, 12). Three of the patients had an epicardial electrode, while the indifferent electrode and pacemaker were implanted subcutaneously as described above. Two of the patients had their pacemaker and indifferent electrode implanted in the axilla. Ventricular synchronous pacemaker treatment was established, with the endocardial electrode functioning as both detector and stimulator, (8) and atrial synchronous pacing was effected by means of detector electrode inserted by mediastinoscopy close to the posterior aspect of the atrium (13).

Technical Description of the Pacemakers

The pacemakers (EM 139, EM 142, EM 141, EM 143, Elema-Schönander AB, Solna, Sweden) used in this study (Fig. 1) were manufactured, with minor modifications, from May 1964 (EM 139) until August 1968. All of them induce biphasic stimulating impulse, whereby the integral of current through the patient is zero.

Fixed rate pacemakers

Two pacemakers of fixed rate type have been used (EM 139 and EM 142). These pacemakers differ only in that EM 139 has a battery consisting of five Hg cells, and EM 142 of four Hg cells. The frequency-determining generator consists of a multivibrator with complementary transistors (V1 and V2, Fig. 2). This operates a transistor (V3), which switches the impulse energy to the electrode system. The impulse energy is taken from a charged capacitor (3.3 μF, previously 4.7 μF), which is charged by the entire battery voltage in the intervals between outgoing impulses. The duration of the impulse is about 2 ms, and the rate is usually about 70 beats/min. The peak amplitude of the impulse is 6.7 V in EM 139 and 5.2 V in EM 142.

Synchronous pacemakers

Two types of such devices have been used, and their basal construction is shown in Fig. 3.

Ventricular synchronous (EM 143). The same electrode system is used for stimulating (monopolar endocardial electrode and indifferent electrode) as for picking up the spontaneous QRS complex of the patient. If these complexes have a rate frequency above the basal rate of the pacemaker the impulses emitted are synchronized so that they fall within the absolute refractory period. In other words, the pacemaker runs with the heart rhythm without giving off active impulses. Should the patient' spontaneous heart rate fall below the pacemaker's basal rate, it emits active impulses which maintain the heart rate at the desired level. Should tachycardia or disturbances occur above the maximal synchronous rate, the signal rate is divided by 2, 3 and so on, so that the pacemaker emits impulses within the interval which is determined by the basal and the highest synchronous rates.

Atrial synchronous (EM 141). With this type of pacemaker an additional electrode is used to pick up the atrial complex. Block C (Fig. 3) in this case functions as time-measurement circuit which simulates the PQ interval. In other respects this pacemaker functions in the same manner as the ventricular synchronous.

The fixed rate pacemaker and the basal frequency

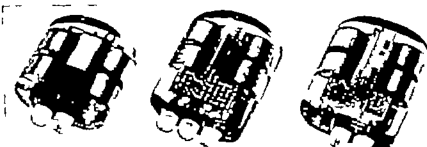


Fig. 1. Pacemakers EM 142, EM 141 and EM 143 (from the left).

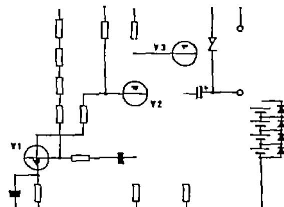


Fig. 2. Circuit diagram EM 142.

generator in synchronous pacemaker need voltage corresponding to 2 Hg cells to act as pulse generator. With further decline in voltage, pulse generation ceases.

Electrodes

I this study unipolar endocardial electrodes (EMT 588, Elema-Schönander AB) as well as indifferent electrodes (EMT 564, Elema-Schönander AB) have been used almost exclusively. EMT 588 has an electrode tip consisting of platinum cylinder with diameter of 2.5 mm and length of 6 mm, and round spherical end. Its area is about 50 mm². EMT 564 consists f round stainless steel plate of 20 mm diameter. The cable is made of four stainless steel bands wrapped around terylene core and insulated with polyethylene.

A pacemaker electrode system is an electrical dipole lying in relatively homogeneous volume resistance. When voltage is applied on the electrodes it produces an electrical field in this volume resistance (= the patient). This field causes measurable differences in potential. As result it is possible to measure the pacemaker impulse from an ECG lead.

Method of Analysis

Equipment

Until August 1968 all analyses of pacemakers were carried out on oscilloscope (Tektronix 502 A) equipped with polaroid camera for photographing the impulse. Since August 1968 special device for analysing pacemaker impulses (Nickab, Västra Frölunda, Sweden) has been in use initially along with the oscilloscope analysis. Since February 1969 only the new apparatus has been employed. Detailed description of the pacemaker impulse analyser and special views concerning technique of measurement have been reported elsewhere (23).

Methods of measurement

All measurements were carried out with the patient lying down and breathing normally. Connection with the measurement apparatus was usually effected by means of standard lead III. In patients in whom the pacemaker and the indifferent electrode were located in the axilla, lead I was used because in these cases the largest amplitudes appear in this lead.

Observations

The pacemaker analyses were initiated by continuous observation of the ECG for at least couple of minutes. Then the pacemaker impulses were analysed as regards pacemaker rate, peak amplitude (millivolt), pulse duration (ms) and decay ratio, expressed as the relation between peak amplitude and the amplitude measured 0.4 ms after the beginning of the impulse. Since the new pacemaker analyser came into use, this parameter has been expressed as decay time of the impulse, measured as the time constant for simple capacitor discharge (ms). These two means of expression can be compared by conversion according to Fig. 4. The parameters examined are shown in Fig. 5.

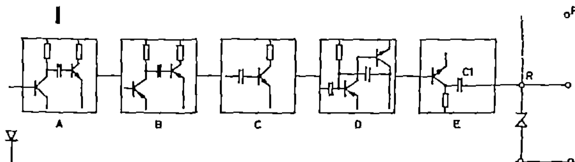


Fig. 3. Block diagram for the synchronous pacemakers. (A) Amplifier with two transistors. (B) One shot flip-flop, A signal defining circuit in the ventricular synchronous pacemaker, a PQ-measuring circuit in the atrial synchronous pacemaker. (C) A synchronous-limiting circuit which limits the highest synchronous rate and eliminates disturbances whose rate exceeds this limit. (D) Free running multivibrator with complementary transistors which can be synchronised to incoming signals. (E) Output switch which connects pulse energy to the patient. Pulse energy is stored in the C 1 capacitor, and the energy source for these pacemakers consists of four Hg cells.

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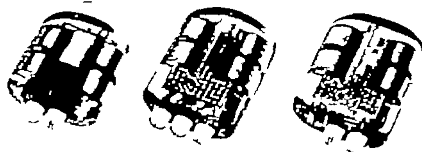


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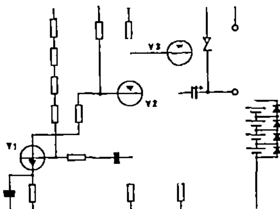


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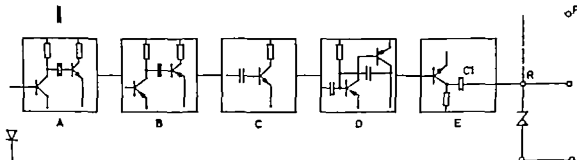


Fig. 3. Block diagram for the synchronous pacemakers. (A) Amplifier with two transistors. (B) One shot flip-flop, A signal defining circuit in the ventricular synchronous pacemaker PQ-measuring circuit in the atrial synchronous pacemaker. (C) A synchronous-limiting circuit which limits the highest synchronous rate and eliminates disturbances whose rate exceeds this limit. (D) Free running multivibrator with complementary transistors which can be synchronised to incoming signals. (E) Output switch which connects pulse energy to the patient. Pulse energy is stored in the C 1 capacitor, and the energy source for these pacemakers consists of four Hg cells.

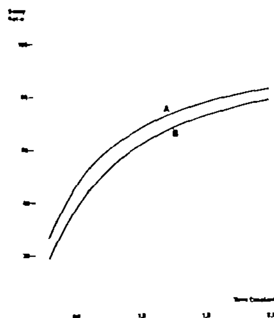


Fig. 4. Relation between decay ratio measured at 0.4 ms (A) and 0.5 ms (B) and time constant.

This method of analysis was employed for both fixed rate and synchronous pacemakers. The synchronous pacemakers were, moreover, evaluated as regards their synchronizing ability. An external pacemaker was used, giving off impulses which were made to trigger the implanted pacemaker via electrodes placed on the skin. The strength of the trigger impulse was increased until it triggered the implanted pacemaker with certainty. The rate of the trigger impulse was then successively raised. With this test it has been possible to assess the maximal synchronous rate of an implanted pacemaker, i.e. while it is still continuing to follow all triggering impulses (9, 31).

In cases in which patients with ventricular synchronous pacemakers have not had basal pacemaker rhythm at the time of the examination, an attempt has been made to decrease the patient's autonomous heart rate below the basal rate of the pacemaker. In many cases basal pacemaker rate has been established by means of massage of the carotid sinus. When the desired result was not achieved in this way, the patient has been given acetylcholine i.v. (50–150 mg) in the manner recommended by Hallén et al. (6). Following this injection a brief period of bradycardia occurs, during which time the pacemaker stimulates the heart. Edrophonium chloride, Tensilon®, is said to give the same result as acetylcholine injections (28), but has not been used by us.

Checking of the pacemaker was carried out for the first time on the day following its implantation. Subsequent checking was done a month or two later and then at intervals of three or four months, while the pacemaker was in use. In the present study pacemakers have been evaluated at slightly more varying intervals in order to gain at least some information each month so as to determine the real need for checking and to obtain values constituting an indication for replacement.

Every patient has acted as his own control. The first findings regarding measured values were used as reference data. Comparisons between the peak amplitude of different patients could be made, notwithstanding differences in the absolute size of directly-measured parameters, as these were determined relatively as a percentage of the original value.

Methodological Studies

In 13 patients variations in peak amplitude resulting from respiratory movements were studied. In these cases pacemaker impulse curves were first measured in maximum inspiratory and maximum expiratory apnoea. The patient then breathed normally and all the pacemaker curves for one minute were exposed to a polaroid camera. The differences in peak amplitude were determined.

Pacemakers of type EM 142 with varying output, corresponding to four, three and two fourths of the normal, were connected to four patients. In this way the usual analysis of impulse curves could be made in the "absence" of one or two of the pacemaker's usual four Hg cells. The real output of the pacemaker was measured at the time of the procedure and then correlated with the analysis of the pacemaker curves. This was done to show the relation between peak amplitude and pacemaker output under otherwise identical conditions.

Time constant, duration and rate are parameters which are in no way influenced by the method of measurement. These are well defined by the pacemaker system. More detailed studies of the measuring procedure of these parameters are therefore unnecessary.

In 33 patients studies concerning the stabilised threshold of stimulation were carried out for the pacemaker electrodes used. The lowest pacemaker voltage which continued to cause regular stimulation of the heart was regarded as the threshold of stimulation. The measurements were carried out with a threshold analyser (EM 365, prototype), the current being reduced by stages of

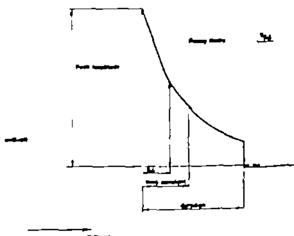


Fig. 5. The stimulation impulse and its parameters.

0.1 V during continuous ECG observation. The threshold was considered stabilized when at least one month had passed following insertion of the electrode (27). As rule threshold measurements were made at the time f pacemaker exchange and are routine feature of this procedure.

Testing of the impulse duration and output of the synchronous pacemakers at varying rate was carried out at Elema-Schönander.

All explanted pacemakers were tested regarding their performance and function. In most cases these measurements were made immediately after explantation at Falun Hospital, and also subsequently at the pacemaker laboratory of Elema Schönander. For various reasons connected with transport damage, some f the pacemakers could be tested at only one place.

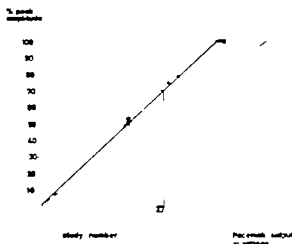


Fig. 6. Relation between peak amplitude (PA) and output voltage of the pacemaker EM 142. (Dashed line represents the expected relation for five-cell pacemaker EM 139.)

RESULTS AND COMMENTS

Equipment

Both kinds of equipment employed for pacemaker checking proved reliable. Oscilloscopic analysis however was complicated and time-consuming. The special pacemaker analyser on the other hand, proved both faster and simpler to use. More important advantages of the new apparatus were that checking of synchronisation properties of the ventricular and atrial synchronous pacemakers, as well as checking of the basal rate of the ventricular synchronous components could be done in a simple way. These tests were much more difficult to carry out by the oscilloscopic method. For further details see Rydén et al. (23). There were no differences between measurements made in parallel with the oscilloscope and th pacemaker impulse analyser.

Measurement technique

Recording from the extremities, which may be regarded as equipotential, makes the placement of points of measurement irrelevant. The result of the study undertaken to demonstrate the relation between pacemaker voltage and peak amplitude is shown in Fig. 6. The peak amplitude for a pacemaker of type EM 142 with four Hg cells was set at 100%. The amplitudes measured with three and two Hg cells were determined as a percentage of the normal value. As would be expected, decline in function of a Hg cell in a pacemaker of type EM 142 will correspond to a decline in peak amplitude of 25% and for a pacemaker of type EM 139 of 20%.

Various factors which alter the interlocation of the pacemaker electrodes, i.e. which influence the direction of the dipole in the body will also influence the measured peak amplitude. In this connection the chief factors are body position and respiratory movements. The first factor can be avoided almost entirely by making all measurements with the patient lying in the same position. It has been noted that all patients have a lower peak amplitude after a maximum inspiration than after a maximum expiration. Differences in amplitude also occur with normal breathing, but are much less marked. If with both types of breathing, the highest measured peak value is set at 100% and the lowest value is reckoned as percentage of the highest, the differences shown in Fig. 7 are obtained. In individual cases the difference in the recording between maximum inspiratory and expiratory apnoea may be so great that the peak amplitude decreases to 75% of the original value. Conversely differences with normal breathing in no case exceed 10% (mean 6%). Differences of this degree are of less importance for test reproducibility since loss of one or more pacemaker cells, as earlier mentioned, causes very much greater peak amplitude deflections.

Other factors which may change the direction of the dipole in the body are the position of the indifferent electrode. Patients included in this study all had a separate indifferent electrode

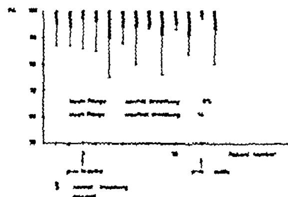


Fig. 7. Relative variations of peak amplitude (PA) at breathing movements (normalized to the maximal observed PA).

placed close to the pacemaker in the subcutaneous fat. No major changes in position should have occurred after healing, although significant shifts in position have been reported for this type of pacemaker by Larsson and Gryte (14). This problem of pacemaker analysis has not arisen, although the small variations in peak amplitude observed may have been influenced by such factors.

An example of the range of the relative peak mplitudes during the observation time for pace-...rs of type EM 142 is shown in Fig. 8. The amplitude for defective explanted pacemakers is also shown. It is evident that, in spite of the factors which produce spontaneous variation of peak amplitude, the variation is clearly less than the differences produced by exhaustion of Hg cells. Peak amplitude may thus be considered as a usable parameter for pacemaker checking if measurements are performed in the above described manner. Peak amplitude and duration vary to some extent with the rate of the synchronous pacemakers. For this reason, in the analysis of such impulse curves it is necessary to note at which rate the check is being made. Fig. 9 demonstrates the extent of the variation.

Values indicating impending pacemaker failure

The values considered as an indication for exchanging the pacemaker are shown in Table II. Peak amplitude is permitted to fall to 70% of its initial value before exchange. According to Fig. 6 the output should then be about 3.7 V if the pacemaker originally had 4 Hg cells (5.4 V EM 142). The stimulating threshold studies are shown in Table III. The highest threshold was 3.0 V (two patients), lowest 1.0 V (one patient), and the mean was 1.9 V. The threshold for two tested epicardial electrodes was 2.5 and 2.. V after four and five years of use, respectively. In most cases these values were determined at the time of pacemaker explantation, which was done under local anaesthesia. It may therefore be assumed that the patient was in a stress situation and probably under adrenergic influence. The measured thresholds may therefore be somewhat higher under normal circumstances. It is also known that variations up to 50% of the threshold value may occur over a 24-hour period (21). In pacemaker therapy it is important that the safety factor, i.e. the relation between the pacemaker output and stimulation threshold level, is sufficient. Sowton (29) states that the optimal energy factor is between 2 and 4. A factor below 2 is believed to constitute a risk of intermittent stimulation. As ten out of 33 patients had thresholds between

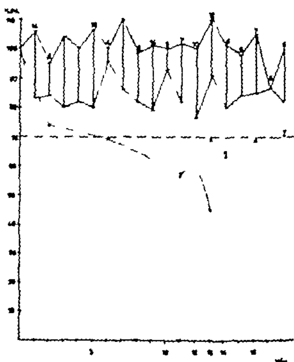


Fig. 8. Distribution of relative peak amplitude (PA) during observation time for pacemakers of type EM 142. (Dashed line represents pacemaker which should have been explanted at six months but was left in position for experimental reasons.) Figures indicate number of observations.

2.1 and 3.0 V at the time of measurement, we consider that the loss of one or more cells from a pacemaker with four Hg cells (EM 142) is incompatible with reasonable safety requirements. In pacemakers with five Hg cells, one, but not two, may become defective before replacement is recommended. In order to meet these requirements, the relative peak amplitude theoretically should not fall below 75% for pacemakers of type EM 142. Since such a restricted limit may lead to unnecessary replacement because of the above-mentioned variations in the measured values (Fig. 8) we have set the limit at 70%. For pacemakers of type EM 139 the limit should for theoretical reasons be set at 60%. The 70% limit has, however, been considered suitable for two reasons. The first is for the sake of uniformity. This eliminates the risk of error. The second is that an exhausted Hg cell sometimes becomes repolarised by the current flowing through the cell. In consequence pacemaker voltage may become less than normal for four Hg cells.

The decay ratio/time constant (TC) in the pacemaker is determined by the size of the output capacitor and the impedance of the electrode and is calculated in the manner described on page 529. The electrode impedance in one individual with one particular pacemaker does not vary much. Significant differences occur primarily when the cable insulation is defective (TC de-

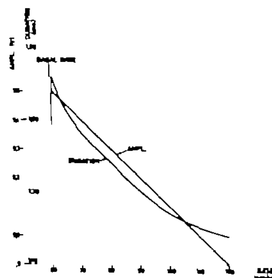


Fig. 9. Duration and peak amplitude as function of the rate in pacemaker EM 133.

Table II. *Values indicating impending pacemaker failure*

Factor observed	Value indicating failure
Rate	Fixed rate: variation > ± 3 beats/min Synch.: basal rate variation > ± 3 beats/min Abnormal response when synchronization ability tested
Peak amplitude	< 70 of the original value
Impulse duration	Variation > ± 0.1 ms
Decay ratio[a]	Variation > ± 10 units (?)

[a] Expressed as decay ratio at 0.4 ms. For explanation see text and Table IV.

Table III. *Stabilized threshold of stimulation for the electrode EMT 588*

Threshold in voltage	No. of pats.
1.0–1.5	11
1.6–2.0	12
2.1–2.5	6
2.6–3.0	4
Total	33

creases), and with cable breakage (TC increases). Table IV shows the mean values of these parameters for different types of normally functioning pacemakers and electrodes which have been used. The highest and lowest mean values in the groups studied are also included. With some combinations only one or two observations have been made, and the values given must be considered to be hypothetical. During the period of observation each patient showed variations in TC (time constant). These have been calculated as differences above and below the mean value for the patient. The largest differences were −5 and +6% units from the mean. No differences were found between various types of pacemakers and electrodes in this respect.

In the pacemakers used, duration is a very constant parameter but changes both with absolute battery voltage and with battery impedance. The main change in the impedance of the battery occurs when one of its cells begins to lose its stable voltage. Impedance changes may occur before this, however. In view of the stability of these parameters as regards duration, the range of ac-

Table IV *Decay ratio and time constant with normally functioning pacemakers*

Pacemaker/Condenser µF	Stimulating electrode[a]	Decay ratio ( ) Mean	Range	Time constant (ms) Mean	Range	No. of pats.[b]
EM 139/4.7	En	73	68–78	1.26	1.04–1.60	6
EM 139/4.7	Ep	—	66	—	0.96	1
EM 139/3.3	En	66	57–73	0.96	0.71–1.27	8
EM 139/3.3	Ep	—	52	—	0.60	1
EM 142/3.3	En	65	62–73	0.93	0.84–1.27	25
EM 142/3.3	Ep	—	55–62	—	0.67–0.84	2
EM 143/3.3	En	62	55–66	0.84	0.67–0.96	7
EM 14 /3.3	En	—	77	—	1.52	1

[a] En = endocardial. Ep = epicardial.
[b] Total number of patients in this Table does not correspond with total number of patients in the material since some of them used two types of pacemakers, or two pacemakers of same type.

ceptable changes in this parameter could be made quite narrow, i.e. ±0.1 ms.

The rate of the pacemakers (basal rate) is very stable. Hence even slight changes indicate that the unit is defective. For the sake of the greatest possible safety narrow limits have been chosen for variations in rate, namely ±3 beats/min.

Some reservations must be made as regards synchronous pacemakers analysed at rates above the basal in respect of duration and peak amplitude. These have been evaluated by studying the in parameters with pacemaker rate and e shown in Fig. 9. Correction of analysis values should be done with this in mind. However, analysis at basal rate is recommended at least in all questionable cases.

Results of pacemaker checking

Type EM 139. A total of 17 pacemakers of this type have been exchanged, including 11 before they had given 18 months service. Data on these 11 pacemakers are shown in Table V a. Of the six pacemakers exchanged at 18 months only two were intact, while the others, both at pacemaker impulse analysis before the exchange and on testing of the pacemaker itself afterwards showed evidence of decline in voltage. None of these patients had subjective symptoms of faulty stimulation, and the ECG showed regular pacemaker rhythm. Of the pacemakers exchanged prematurely one should have been exchanged even without further analysis (no. 6) because of a rise in rate to +6. One pacemaker had to be exchanged as an emergency (no. 2) because the patient developed symptoms in the form of an Adams-Stokes attack. This, however, occurred before values indicative of impending pacemaker failure were found. Today this pacemaker would have been exchanged before clinical symptoms occurred. When checked at ten months this pacemaker showed peak amplitude at 63% and duration +0.2 ms. With regard to previously defined requirements for safety factors, none of the pacemakers which were removed prematurely can be considered to have been exchanged unnecessarily. As regards pacemaker no. 4 this was removed very early partly in order to determine the relation between peak amplitude and pacemaker voltage. With present requirements it would have functioned for a longer period.

Type EM 142. A total of 31 pacemakers of this type have been exchanged (Table V b). Twenty-three of these were removed before the end of 18 months, and eight were exchanged as a matter of routine. Of the latter six were intact and had given normal analysis values before explantation. Two of the pacemakers showed erroneous analysis values, and this finding was confirmed on testing after removal. None of these patients had clinical symptoms, and the ECG showed regular pacemaker rhythm at the time of the pacemaker exchange. Of the pacemakers exchanged prematurely two (nos. 2 and 15) should have been exchanged because of variation in rate, +4 and −4/min, respectively even without impulse curve analysis. Neither of these two patients had subjective symptoms but were picked up at the time of pacemaker check. In three cases the patients were seen at the hospital because of symp-

Table V*a, b c* *Prematurely exchanged pacemakers*

Interval between implantation and exchange reasons for exchanging, and results of the analysis of the impulse wave, and the checking of the pacemakers after explantation.
PA = peak amplitude D = impulse duration in ms. A–S = Adams–Stokes attack RP = regular paced rhythm IM = intermittent stimulation. AV III = atrio-ventricular block of the third degree SR = sinus rhythm. c = mercury cell. p–m = pacemaker Clin = clinical signs. Imp = impulse

No.	Time (mo.)	Reason for exchange	Results of analysis: PA	D ms	Rate	Clin	ECG	Pacemaker check
V*a. Pacemaker type EM 139*								
1	11	PA D	52	+0.2	+3	—	RP	2 0 V
2	L.	Clin	42	+0 4	+2	A S	IM	Diode failure short circuit
3	12	PA D	47	+0.2	±0	—	RP	2 0 V
4	12	PA	78	±0 0	−1	—	RP	1 0 V
5	12.5	PA D	60	±0 0	−1	—	RP	2 0 V
6	13	Rate	76	±0.0	+6	—	RP	Electrolyte leak from one cell gave short circuit
7	14	PA D	39	+0.3	+2	—	RP	3 0 V Fissure in epoxy resin
8	15	PA	68	±0 0	−1	—	RP	Damage at explantation. Values indicate 2 0 V
9	15	D	78	+0.3	±0	—	RP	2 0 V
10	16.5	PA D	54	+0 4	+1	—	RP	3 0 V
11	17	PA D	69	+0.2	−1	—	RP	2 0 V
V*b. Pacemaker type EM 142*								
1	8.5	Clin. No p–m imp.	—	—	—	A–S	AV III	Electronic component failure Transistor V 3
2	10	Rate D	105	+1.3	+4	—	RP	1 c −0 2 V
3	11	PA D	57	+0 5	+1	—	RP	1 −0 3 V
4	11	PA D	60	+0.4	−1	—	RP	1 0 V
5	11.5	Clin. No p–m imp	—	—	—	Tired	AV III	4 0 V Mechanical and electronic components normal
6	11.5	PA D	64	+0.2	±0	—	RP	1 −0 2 V
7	12	PA D	55	+0 2	+2	—	RP	1 0 V 1 −0 8 V
8	13	PA D	45	+0.3	+3	—	RP	2 0 V
9	13	D	100	+1.6	±0	—	RP	1 0 5 V
10	13	D	75	+0.4	+1	—	RP	No values available
11	13	D	73	+0.2	±0	—	RP	1 0 V
12	14	PA D	64	+0.2	+2	—	RP	1 0 V
13	14	PA D	63	+0 4	±0	—	RP	1 0 V
14	15	PA	54	±0.0	−1	—	RP	1 −0 9 V
15	15	Rate PA D	39	+1.0	−4	—	RP	1 0 V 1 0 2 V
16	15.5	PA	52	±0.0	±0	—	RP	1 −0.2 V
17	16	PA	70	±0.0	−1	—	RP	1 0 V
18	16	Clin. No p–m imp.	—	—	—	Tired	AV III	3 0 V Mechanical and electronic components normal
19	16 5	PA D	50	+0 8	−2		RP	2 0 V
20	17	PA D	66	+0.3	±0	—	RP	1 0 V
21	17	PA	68	+0.1	±0	—	RP	1 0 V
22	17	PA D	60	+0.2	−1	—	RP	1 0 V
23	17	PA	57	±0 0	−1	—	RP	1 0 V
V*c. Pacemaker type EM 143*								
Results of pacemakers check after explantation								
1	13	PA D	70	+0.5	−1	—	RP	1 0 V
2	14	D	80	+0.3	−2	—	RP	1 −0.2 V
3	14.5	PA D	66	+0.4	−3	—	RP	1 −0.2 V
4	16	Synchronization ability lost	100	±0.0	−3	—	RP/SR	Electronic component failure in block C (Fig. 3)
5	17	Rate PA D	96	±0 0	−5	—	RP	P-m check normal except basal rate Change not analyzable

toms arising from defective pacemakers (nos. 1, 5 and 18). One of these patients (no. 1) developed Adams-Stokes attacks and was admitted as an emergency case, while the other two complained only of fatigue. Neither of them felt the need to attend the emergency clinic, but waited for the usual pacemaker check.

In all three cases no pacemaker impulse could be seen on the ECG and no impulse curve could be obtained on routine analysis. In case 1 there was a transistor defect in the pacemaker which resulted in a sudden interruption of function. In case 5 all four Hg cells were found to have ceased functioning, although the other pacemaker components were in good condition. In this patient all measured parameters were normal at the time of checking three months earlier (peak amplitude 100%, rate ±0, duration ±0.0, decay ratio +2%). In the last case (no. 18) the course was identical with the previous one (no. 5). Normal measurement values on checking three months earlier were also found in this patient (peak amplitude 91, rate +1, duration ±0.0, decay ratio +3%). Of the 18 pacemakers which were exchanged because of findings on analysis of the impulse curves, none can be said to have been changed unnecessarily for the same reasons as ... eviously adduced for pacemaker EM 139.

Type EM 143. Ventricular-synchronous pacemakers have not been in use as long as fixed rate pacemakers. For this reason, in our study only seven units have been exchanged, two of which functioned for 18 months. Both of these were intact at the time of explantation, although one was not triggered by a negative pulse wave. The findings were negative on clinical examination, ECG and pacemaker impulse analysis in both patients. Data of the remaining five pacemakers are shown in Table V c. One of these (no. 5) was exchanged because of the decline in rate. One pacemaker (no. 4) was found to have normal impulse wave analysis and normal basal rate. However, a check showed that the pacemaker was not normally triggered by the patient's autonomous heart beats, nor was it possible to make the pacemaker increase its rate by use of an external trigger pulse, which had previously been feasible. The other three pacemakers were exchanged on suspicion of battery failure. None of the five patients had any symptoms, and all had normal pacing at the basal pacemaker rate. No pacemaker can be regarded as having been exchanged unnecessarily.

Type EM 141. This series includes only one pacemaker. It was exchanged after 14 months while still functioning adequately. The indication for exchange was the recommendation of the manufacturer, as this type of pacemaker was still relatively new at that time.

Electrode defects

No electrode breaks or defects in electrode cable insulation occurred in the present series.

DISCUSSION

The operational safety of pacemakers has increased, but the literature contains numerous reports on defective functioning of implanted pacemakers even before expiry of the calculated and recommended life of the apparatus (27, 32). Edhag (4) has reached the same conclusion and has described a series in which the same pacemakers and electrodes were used as in this study. Moreover, in several cases of sudden death the pacemaker was found to have been defective (4, 7, 25). In view of these findings a need obviously exists for adequate checking of pacemaker-treated patients. As regards external units the testing of function is simple, since they are easily accessible for direct analysis. For implanted pacemakers different methods have been used to reduce the risk of suddenly occurring stimulation defects. One method, originally described by Lillehei et al. (15), is based on roentgenological examination of the pacemaker batteries. This method can be used only for certain types of pacemakers (e.g. not for those employed in this study) and, according to a recent report, seems to be unreliable (18). Other methods are restricted to use in conjunction with particular makes of pacemaker (20). It is, moreover, undesirable to use methods which, however elegant they may be, are laborious and time-consuming (3). Given the requirement of the fullest possible analysis of pacemaker data with a reasonable examination, it would appear that oscilloscopic analysis of the pacemaker impulse curve is at present the best method available. Different authors have described various ways of perform-

ing this type of analysis. Emmrich and Kraft (5), for example, as well as Paeprer et al. (19), place the electrodes on the thorax and above the implanted pacemaker This, however leads to difficulty in evaluating the peak amplitude. Electrode placement, even with minor shifts of position between one examination and another will affect this amplitude a circumstance also noted by these investigators, who therefore prefer to use impulse duration for checking. It is still better to employ standard ECG leads, in regard to which our experience, as well as that of others (2, 10 29) shows that adequate deflection can always be obtained provided that unipolar pacemaker electrodes are employed and a suitable ECG lead is selected.

Although several articles have been written on oscilloscopic analysis of pacemaker impulse curves, as mentioned in the introduction, no coherent studies have been carried out based on uniformly treated and checked patients. Such series, however need to be reported for correct evaluation of the method used for checking. Moreover pacemakers of different manufacture have different characteristics. This must be kept in mind when establishing the values indicative of impending pacemaker failure and is an important reason for continuing studies of this nature.

In our opinion the values we have used to indicate impending pacemaker failure have proved satisfactory Of the patients who developed symptoms as the result of pacemaker failure, one, under present conditions, would have had his pacemaker exchanged in time. The transistor defect could not have been revealed by any kind of *prophylactic check. The two pacemakers* which within the space of three months showed loss of function in three and four Hg cells are particular worthy of note. It is possible that these pacemakers could have been explanted before clinical symptoms occurred if checking had been more frequent. On the other hand, more frequent checking would hardly be feasible.

No pacemaker was unnecessarily exchanged if one accepts the safety requirements which we consider indispensible. Only eight of the 56 pacemakers would have been exchanged at the actual time if it had only been possible to rely upon the pacemaker rate, ECG and clinical findings.

Probably the final result would have turned out to be a larger number of acute episodes or symptoms than in four out of 56 exchanged pace makers if checking of the kind described had not been undertaken. This statement is made in view of the results of the final pacemaker testing after explantation, and the fate of the only patient where notwithstanding the values of the pacemaker check, the pacemaker was not exchanged. In the entire series of pacemaker cases treated at Falun Hospital no death occurred in which electrode dislocation or defect of the electrode or pacemaker was the sole or a contributory cause (24).

The method used has further advantages. It permits the classification of cases of stimulation failure. Such cases may include pacemaker failure, electrode failure (breakage or defective insulation), electrode dislodgement and exit block. Of these, the method does not directly detect dislodgement and exit block. If, however the impulse analysis is normal and the roentgenogram shows that the electrode has not moved, prednisolone may be tried. The stimulus threshold then falls within a short time and if the pacemaker impulses become effective, this would suggest exit block (20 21) as the cause of the stimulation failure. A more direct method of studying possible risks of exit block is successively to reduce the pacemaker output voltage in the manner described by van den Berg and Thalen (1), Preston et al. (20) and Schmutzer (26) This procedure, however is impossible with the type of pacemaker used by us.

Since the average age of pacemaker treated patients is high, it may be expected that dizziness and syncopal attacks and similar symptoms will *occur e.g. of cerebral origin. The history in these* conditions may suggest symptoms of pacemaker failure. In such cases pacemaker checking constitutes a valuable diagnostic complement.

Still another reason for considering the method to be of value is that it gives the patient a feeling of confidence, which should increase as more information is provided about the function of the pacemaker and electrode system at the time of the check-ups. As regards economic factors the pacemaker-checking equipment is inexpensive in comparison to the high costs involved in pacemaker therapy In the future it is our intention to exchange each pacemaker when it shows signs of failure. In this study 11 of the 56 pacemakers explanted were intact at the time of routine ex

change as regards both impulse analysis and subsequent testing after explantation. They could have functioned for a longer period, which would not only have spared the patients unnecessarily frequent procedures but would have reduced the expenditure on pacemakers. Thus, when the total number of pacemaker-treated patients is large, the saving of a month or more on individual patients may result in considerable economies.

Among the parameters analysed we consider peak amplitude as essential as impulse duration for revealing impending failures. This defect is usually associated with simultaneous decrease in peak amplitude and increase in impulse duration. In this series, however, there were several cases in which only one of these parameters had a value outside the accepted range. We are therefore unable to concur with the statement of Paeprer et al. (19) that pulse duration alone is sufficient for purposes of checking. We also consider that the tolerance of 30% increase in impulse duration suggested by these authors, is too wide for the pacemakers in question. The reason why impulse duration may be prolonged while peak amplitude is normal is probably that analysis happened to be performed just when the internal resistance in the battery had risen immediately before it ceased functioning. However, functional loss of Hg cells without this increase in impulse duration may occur, as was noted also by Wanjura et al. (33). In our experience these cases show a decline in peak amplitude. Thus, in the event of an isolated instance of pulse duration or peak amplitude decrease, a delay of one or more months would, we believe, involve a risk of stimulation failure.

As expected, the rate for this pacemaker proved extremely stable. When the patient is able to take his own pulse, this is the simplest and indeed the only method of continuous pacemaker checking at home. However, we have noticed that, notwithstanding instruction, many patients are unable to manage this. In some cases relatives can help, and a transistor radio may be used to make a distinctive click with each pacemaker pulse (16).

In our series the decay ratio/time constant yielded no positive findings. The electrode system was very reliable, but Edhag (4) reports a few cases of defective insulation, probably arising from adjustment of the electrode cable. Apart from the examining of patients with stimulation failure we consider it justified to check this parameter after every surgical procedure involving risk of damage to the cable insulation. That these measurements may also be of clinical value in other circumstances is shown by other authors (17, 25).

In checking synchronous pacemakers attention should be paid to the previously mentioned variation in peak amplitude and impulse duration with changing pacemaker rate. In doubtful cases, checking at the basal rate of the pacemaker should be carried out. In each pacemaker of this type it may be desirable at the first examination to carry out the impulse analysis at a few different rates, including at least the basal and maximum synchronous rates, in order to obtain some idea of its specific variations.

Table V, *a*, *b* and *c*, shows clearly that by far the most common cause of prophylactic pacemaker replacement is the too rapid exhaustion of Hg cells. This is due to the fact that these cells in no way measure up to the data provided by the manufacturer. Some cells are already exhausted when only 35% of stated capacity has been used. In one case (Table V*a*, no. 6) there occurred what was probably a primary cell failure, which caused a break in the epoxy resin, which in turn caused two secondary cell failures. Electronic defects occurred in three cases (*A*, Table V *a*, no. 2; *B*, Table V *b*, no. 1; and *C*, Table V *c*, no. 4). These have no common factor. The diode defect (*A*) resulted from mechanical stress arising in the encapsulating process. The transistor defect (*B*) arose from an internal mechanical defect in the transistor, which occurred during assembly by the transistor manufacturer. The last defect (*C*) was in a transistor: one of the transistor parameters changed, so that it lay outside the specified range, with the result that it ceased to function.

Examination of a pacemaker-treated patient should be carried out within 24 hours of implantation, when the pacemaker appears to be working satisfactorily and has definitely reached the body temperature. A second examination should be made one month later. The value obtained from these analyses then form the basis of further examinations, which, at present, we perform five and ten months later. Subsequent analyses are carried out at 3-month intervals during the remaining life of the pacemaker. These intervals, which have been used during the past year are

based on experience which shows that hardly any defects occur during the first year of service. Where any doubt exists as to the analysis values, the examination should be repeated two or three weeks later. Each examination should include a careful clinical evaluation of the patient as part of the pacemaker analysis. Like Siddons and Sowton (27) and Sowton (29) we incline to the view that pacemaker-treated patients should be checked by specialists familiar with the method of treatment, preferably in a pacemaker clinic.

It should, however, be forcefully pointed out to the patients that it is most important to contact the hospital at times other than those appointed for testing, should they have any symptoms or change in pulse rate beyond the ordinary. These symptoms need not be very striking, as evidenced by the fate of two of our patients (Table V *b* nos. 5 and 18) who failed to follow instructions.

The pacemaker checking here described will be further developed for the pacemakers in the 150-series (Elema-Schönander AB) which are now being produced. As their electrical characteristics are somewhat different, the normal range of values must be altered somewhat. The range of variation of the duration should provisionally be set at 0.3 ms for EM 151 and EM 152, and at 0.5 ms for EM 153, EM 155 and EM 156. The change in rate can be fixed at 3 beats/min. For the moment these values may be considered hypothetical. For further safety the construction of EM 151 and EM 152 will be changed, so that when a Hg cell is exhausted, the pulse rate will fall by about 10 beats/min. Impulse duration will not be significantly influenced by the condition of the Hg cells. It is our intention to carry out further studies by the methods described, but we hope that future pacemakers will be so constructed that they give unmistakable signals—e.g. concerning cell failure—which would further simplify pacemaker checking.

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TETANUS ANTITOXIN PRODUCTION AND GAMMA GLOBULIN LEVELS IN PATIENTS WITH CIRRHOSIS OF THE LIVER

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Abstract. Antibody response to tetanus toxoid in patients with hepatic cirrhosis and in controls has been compared. No significant difference can be demonstrated. The antibody responses in twenty-four patients with hepatic cirrhosis are compared with the serum concentrations of γG, γA and γM at the time of the second injection. No relationship can be demonstrated between antibody response and gamma globulin level. These results would favor the hypothesis that the high gamma globulin concentration found in hepatic cirrhosis is not due to an increased immunologic potential, but rather to an increased antigenic influence.

The high serum concentration of gamma globulin in patients with cirrhosis of the liver is due to an increased rate of synthesis (1). All gamma globulin is antibody and so the increased synthesis must be due either to increased antigenic influence or to increased immunologic potential. The latter explanation has been generally accepted after the work of Havens et al. in 1951 (4). The authors demonstrated that a booster dose of diphteria toxoid given to Schick-negative patients provoked a higher antibody response in patients with cirrhosis than in control patients with certain acute and chronic diseases. Havens et al. (5) later studied this problem using tetanus toxoid as the antigen. They demonstrated that a booster dose of this antigen given to previously immunized patients with hepatic cirrhosis produced an unusually high response in 20% of the patients. There were no controls in this study.

Cherrick et al. (3) failed to confirm these observations using immunization with tetanus toxoid in patients with hepatic cirrhosis. They mention the possibility that the toxoid used in their experiments was not a very potent one. However individual rises in tetanus antitoxin titers of high degree were demonstrated in a few subjects.

Paronetto and Popper (9) studied antibody production experimentally in mice with acute and chronic liver injury produced by carbon tetrachloride or allyl alcohol. Heterologous red cells or sterile horse serum were used as antigen. They found that liver injury enhanced the antibody response in both types of experiment. They conclude that the liver injury seems to have an adjuvant effect.

The question of antibody response in patients with hepatic cirrhosis is still not settled. We have therefore studied this problem using adsorbed tetanus toxoid, known to be a potent vaccine, as the antigen.

MATERIAL AND METHODS

Only patients with cirrhosis confirmed by biopsy were studied. Controls, with chronic diseases other than cirrhosis, were matched according to age and sex with the liver patients. Twenty-seven liver patients and twenty-eight controls were studied. Mainly because of high mortality in cirrhosis large number of patients were lost during the experiment. A second group of nine patients with cirrhosis of the liver was therefore included later (nos. 16–24 in Table I).

Prior to the second injection, the gamma globulin concentration in the sera of the cirrhosis patients was determined by paper electrophoresis according to the method of Laurell et al. (7). In most of the cases with cirrhosis the concentration of γG, γA and γM was also determined according to Mancini et al.'s method (8). The vaccine was purified tetanus $Al(OH)_3$ adsorbed toxoid from the State Serum Institute, Copenhagen. The intervals between the injections of vaccine were four weeks between the first and the second injection, and

Table I. Titers of tetanus antitoxin in log units per ml in cirrhosis patients at different stages of tetanus immunization. All < −3.50 AU/ml prior to the 1st injection

Pat. no.	4 weeks after 1st inject.	2 weeks after 2nd inject.	5–11 months after 2nd inject.	2 weeks after 3rd inject.
1	< −3.50	−1.52		
2	< −3.50	< −3.50		
3	< −3.50	< −3.50		
4	−3.50	−3.26	−_.89	+0.07
5	+1.16	+1.19		
6	−1.71	−0.82		
7	< −3.50	−1.73	−1.11	−0.40
8	< −3.50	−3.27	−2.37	−0.97
9	−3.37	+0.03	−0.65	−1.22
10	< −3.50	−1.16		
11	−0.99	−0.59		
12	−3.06	−1.23	−1.47	+0.03
13	< −3.50	< −3.50		
14	< −3.50	< −3.50		
15	+0.51	−0.72	−0.30	0.28
16	−3.11	−0.64	−0.14	0.64
17	< −3.50	−1.31	1.22	+1.11
18	−3.50	−0.80	−1.00	+1.67
19	−_.33	−0.62	−0.59	+1.44
20	< −3.50	−3.28	< 3.50	−1.83
21	−3.50	−0.85	−1.43	−1.44
22	< −3.50	−0.62	−0.91	−1.11
_3	−2.27	−0.07		
4	< −3.50	−1.65		
otal	24	24	13	13

Table II. Titers of tetanus antitoxin in log units/ml in control patients at different stages of tetanus immunization. All < −3.50 AU/ml prior to the 1st injection

Pat. no	4 weeks after 1st inject.	2 weeks after 2nd inject.	5–12 months after 2nd inject.	2 weeks after 3rd inject.
1	< −3.50	−1.73		
2	−2.09	−0.30	−0.24	+0.87
3	< −3.50	< −3.50		
4	< −3.50	−1.03	−0.86	
5	−3.17	−0.05	−0.26	+0.71
6	< −3.50	< −3.50		
7	< −3.50	< −3.50		
8	< −3.50	< −3.50		
9	−2.74	−0.77		
10	−0.08	+0.06	−0.29	+0.20
11	< −3.50	< −3.50	< −3.50	−0.13
12	< −3.50	< −3.50		
13	−3.50	−0.85	−0.84	+1.09
14	< −3.50			
15	< −3.50	< −3.50	−2.34	−0.37
16	< −3.50	< −3.50		
17	−0.04	+0.45	−0.13	+0.88
18	< −3.50	−1.88		
19	< −3.50	−0.77		
20	< −3.50			
Total	20	18	8	7

5–1 months between the second and the third injection. The blood samples ere taken before and four weeks after the first injection, and before and tw weeks after the second and third injection. Titration of the tetanus antitoxin as carried out in mice according to Ipsen method (6). The results are expressed in log international units per ml, −3.50 being taken as the limit for measurable antitoxin. Only patients ith −3.50 prior to the first injection ere included in the study

RESULTS

Tables I and II give the responses to the differ ent injections for the cirrhosis patients and the controls, respectively and the number of pa tients at each stage. The number of the cirrhosis patients was _4 at the second injection, and that of th_ controls 20· th numbers at the blood sampling two weeks later were 4 and 18 The statistical examination revealed that the titers before and after the second injection were not nor mally distributed in either of the groups. Accordingly the means and standard errors have no meaning, and the comparison between the two groups has therefore been made graphically This is shown in Fig. 1 which gives the relation between the individual log titers four weeks after th first injection (abscissa) and two weeks after the second injection (ordinate) The deviation

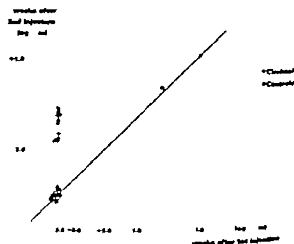


Fig. 1 The relation between tetanus antitoxin in log units per ml in patients with cirrhosis and in contro's 4 weeks after the first injection (abscissa) and 2 eeks after the second injection (ordinate).

Table III *Comparison between mean tetanus antitoxin titers in cirrhosis and in control patients 5–11 months after the 2nd injection, and 2 weeks after the 3rd injection of tetanus vaccine*

	Group	No.	Titer in log/U/ml Mean	Titer in log/U/ml S.E.	Titer difference Mean	Titer difference S.E.
5–11 months after 2nd injection	Cirrhosis	13	−1.16	0.34	0.10	0.56
	Control	8	−1.06	0.44		
2 weeks after 3rd injection	Cirrhosis	13	+0.51	0.24	−0.05	0.41
	Control	7	+0.46	0.33		

from a normal distribution is apparent, inter alia, from the fact that one group of patients starting without measurable antitoxin prior to the second injection has little or no titer increase another group likewise starting without measurable antitoxin shows a pronounced increase, whereas values in between are lacking. The titers in the two groups are similarly distributed and no difference between them can be demonstrated.

Five to twelve months after the second injection there were only 13 patients left in the cirrhosis group, and eight (7) in the control group. The titers in both groups were normally distributed. The means and standard errors of the titers are presented in Table III together with the mean differences and their S.E. The values show clearly that there are only chance variations between the two groups also at this stage. The same is apparent from the even distribution of the points in Fig. 2, which gives the relationship of the individual titer prior and subsequent to the third injection. The possible effect of the matching according to age and sex has been examined. Any such effect should produce a positive correlation between results for patients belonging to the same pair. However no such correlation was found. Furthermore no dependency

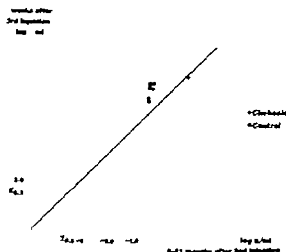


Fig. 2. The relation between tetanus antitoxin in log units per ml in patients with cirrhosis and in controls before (abscissa) and after (ordinate) the third injection.

Table IV *Antitoxin increase in log units after 2nd injection in relation to the concentrations of total gamma globulin, gamma G, M and A (g/l)*

Pat. no.	Antitoxin increase in log units	Gamma total[a]	Gamma G[b]	Gamma M	Gamma A
13	?[c]	32.3	31.1	2.47	6.57
3	?[c]	21.7			
2	?[c]	13.6			
14	?[c]	33.2	30.6	0.54	5.58
5	0.03	19.1	23.1	1.64	
15	0.19	17.7	16.1	1.81	5.36
20	>0.22	24.4	27.1	4.69	3.60
8	>0.23	25.7	24.2	4.92	3.05
4	>0.24	8.4			
11	0.40	35.6	40.3	4.54	6.08
6	0.89	21.1	23.1	0.70	5.20
19	1.71	23.2	27.3	1.92	13.45
7	>1.77	18.1	20.2	1.93	8.01
12	1.83	12.5	17.8	0.30	2.52
24	>1.85	–	19.0	0.95	4.94
1	>1.98	11.9			
17	>2.19	13.6	16.7	0.72	9.10
23	2.20	35.8	30.2	2.84	4.42
10	>2.34	8.6	15.0	1.15	5.15
16	2.47	17.7	22.1	2.74	2.98
21	>2.65	27.6	35.6	1.71	6.24
18	2.70	14.5	21.3	0.98	5.39
22	>2.88	22.1	22.8	3.04	3.82
9	3.40	15.1	22.9	0.71	5.20
Total	24	24	20	20	19

[a] Determined by paper electrophoresis.
[b] Determined according to Mancini et al's method.
[c] Both the prior and the subsequent titers below −3.50.

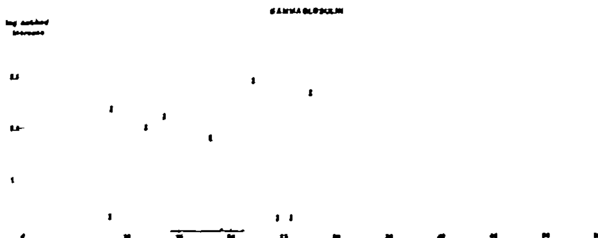


Fig. 3. The relation between tetanus antitoxin increases after the second injection in log units per ml and gamma globulin determined at the same time by paper electrophoresis in patients with cirrhosis of the liver.

of antibody response on age or sex could be demonstrated in either of the two groups. The fact that the female patients with cirrhosis were all non-alcoholics, whilst the men were alcoholics, might be taken to indicate that the alcoholic factor has not influenced the antitoxin response.

In Table IV the increases in antibody titer after the second injection in the cirrhosis patients are given in order of increasing antibody response together with the corresponding values of the total gamma globulin and of gamma G, M and A. The sum of the three immunoglobulins, determined according to Mancini, is generally higher than the total gamma globulin determined by paper electrophoresis. The relationship between the concentration of the different gamma globulins and the antibody increases has been examined graphically in Figs. 3, 4, 5 and 6. These diagrams disclose that no correlation between these values can be demonstrated in our material.

DISCUSSION

Our experiments show that no difference in antibody response can be demonstrated between patients with hepatic cirrhosis and controls. These results are in agreement with the results of Cherrick et al. (3) who used the same method, vaccination with tetanus toxoid, and in contrast to the results of Havens et al. (5) who used vaccination with diphteria toxoid. One explanation of this discrepancy may be that the scattering of



Fig. 4. The relation between tetanus antitoxin increases after d injection in lo its per ml and gamma G globulin determined according to Mancini et al. in patients th cirrhosis of the liver.

antibody increases is so great both in patients and in controls that smaller differences cannot be significantly demonstrated with the limited number of observations in question. Another explanation would, of course, be that there is no increased antibody response in patients with hepatic cirrhosis.

We have not been able to demonstrate any relationship between gamma globulin concentration and antibody response. A positive correlation would be expected if these patients have an increased immunologic potential, not because the tetanus antitoxin would add a measurable quantity of gamma globulin to the serum gamma globulin concentration, but because the gamma globulin concentration might reflect the immunologic potential. The higher the gamma globulin concentration, the higher the immunologic potential. We have not found such a correlation between gamma globulin and antibody response.

In conclusion we have not found that patients with hepatic cirrhosis have a higher antibody response to an antigenic stimulus than controls, and we have not found any relationship between gamma globulin concentration in hepatic cirrhosis and antibody response. These results would favo[r] the hypothesis that the high gamma globulin concentration found in hepatic cirrhosis is not due to an increased immunologic potential, but rather to an increased antigenic influence. This antigen influence may be the antigenic effect of hepatocyte breakdown products (2).

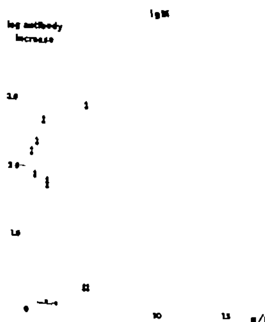


Fig. 5. The relation between antitoxin increases after the second injection in log units per ml and gamma M globulin determined according to Mancini et al. in patients with cirrhosis of the liver.

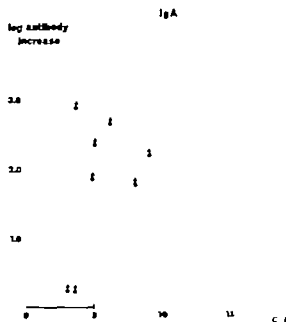


Fig. 6. The relation between tetanus antitoxin increases after the second injection in log units per ml and gamma A globulin determined according to Mancini et al. in patients with cirrhosis of the liver.

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INCREASED RATIO OF GLYCINE/TAURINE CONJUGATED BILE ACIDS IN THE EARLY DIAGNOSIS OF TERMINAL ILEOPATHY

Preliminary Report

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Abstract. In patients with verified terminal ileopathy the ratio of glycine/taurine conjugated bile acids in duodenal contents has been found to be increased. In two patients with diarrhoea and steatorrhoea this ratio was demonstrated to be increased even before conventional methods showed evidence of terminal ileopathy. The diagnosis of Crohn disease localized to the terminal ileum was later confirmed by operative and microscopic findings.

An upper limit of 4.4 for the ratio of glycine/taurine conjugated bile acids (G/T ratio) in duodenal contents was found in 35 subjects examined (2). The subjects had no diarrhoea, steatorrhoea or signs of small bowel disease, there was no significant growth of bacteria in the proximal small bowel, and the subjects had no signs of thyroid disease, nor of disease of the liver or biliary tract.

The upper limit mentioned is in agreement with the values found by some other workers (6, 8, 11). In some materials, however, a few of the so-called normal subjects showed a higher ratio (1, 7, 12, 13) but these subjects were not examined according to the criteria mentioned above.

It has been demonstrated previously that the G/T ratio is increased in patients with jejunocolic fistula (9), with terminal ileitis, and after resection of the terminal ileum (1, 4, 8, 10, 11). In the reports on patients with terminal ileitis published so far the diagnosis had, however, been established by the conventional methods prior to the bile acid analysis.

In the following are presented two patients with diarrhoea and moderate–slight steatorrhoea in whom an increased G/T ratio indicated ileal disease at a time when no other examination focussed attention on the terminal ileum. The diagnosis of terminal ileitis was later confirmed by operative and microscopic findings.

METHODS

Bile acid analysis of duodenal aspirates was performed according to the procedure described by Bruusgaard (3), using 3-hydroxysteroid dehydrogenase, preceded by thin-layer chromatography. The duodenal aspirates were obtained after cholecystokinin stimulation of the gall bladder emptying (1 Ivy dog unit per kg of body weight intravenously).

CASE REPORTS

Case 1

A woman, aged 24, was admitted to hospital because of watery diarrhoea of six months' duration. Immediately before admission she had typical erythema nodosum.

Faecal fat determination showed moderate steatorrhoea (10–13 g fat/day). No pathogenic enteric bacteria or Giardia lamblia cysts were found in the faeces, the Widal test was negative, and there were no demonstrable antibodies against Yersinia enterocolitica in the blood. The pancreatic and hepatic functions, D-xylose absorption and thyroid function were normal. Oral loading with lactose showed normal increase in blood glucose, and no diarrhoea occurred concomitantly. Biopsy from the duodenum was normal. Culture of fasting duodenal aspirate showed no significant growth. Immunoglobulins were normal. By an augmented histamine test normochylia was found. The urine contained no 5-hydroxy-indole acetic acid. Oral cholecystography was normal. The Schilling test with intrinsic factor was normal (9.5% excreted). Serum cholesterol was at the lower normal limit (146 mg%). Proctoscopic examination with biopsy was normal. Barium studies of the small bowel showed no changes in the terminal ileum.

Bile acid analysis revealed G/T ratio of about 13.

The patient was discharged without any special treatment, but readmitted to hospital tw months later because of exacerbation of diarrhoea and colicky abdominal pain. On this occasion barium studies were suggestive of terminal ileitis, and the findings from selective angiography of the superior mesenteric artery were typical of Crohn disease.

As the symptoms persisted despite treatment with prednisone, laparotomy was performed. The gross appearance of the terminal 20 cm f the ileum showed typical Crohn disease, and microscopy f a mesenteric lymph node showed inflammation and epithelioid cell granulomas.

Case 2

A man, aged 37 was admitted to hospital because of watery diarrhoea and loss of weight during three months.

Faecal fat determination showed very slight steatorrhoea (about 7 g fat/day). In the blood an insignificant antibody titre against *Yersinia enterocolitica* was found. The hepatic and thyroid functions ere normal, and so was D-xylose absorption. Culture of fasting duodenal aspirate howed *no growth. Immunoglobulins were normal*. Oral cholecystography was normal. The Schilling test with intrinsic factor was slightly subnormal (6.1% excreted). Serum cholesterol was within normal limits (233 mg%). Proctoscopic examination showed typical signs of non-specific proctitis. Biopsy showed severe non-specific inflammation, and no epithelioid cell granulomas were found. Barium studies were suggestive f a severe rectitis, without involvement of the emaining colon. 7 bowel radiology and selective angiography of the superior mesenteric artery were normal.

Bile acid analysis revealed G/T ratio of about 23.

Repeated barium studies five weeks later suggested terminal ileitis

The patient developed an abscess in the ischio-rectal region, and there was *radiological suspicion f an internal* fistula between the ileum and rectum. Laparotomy was performed, and the gross appearance of the terminal 30 *cm of the ileum showed typical* Crohn disease. The suspected fistula was found The histological picture of the removed ileum supported the diagnosis of Crohn disease, although epithelioid cell granulomas were not demonstrated.

DISCUSSION

The low proportion of taurine conjugated bile acids in patients with terminal ileopathy is probably due to loss of taurine conjugates with resultant deficiency in taurine available for conjugation. Anyhow the G/T ratio can be reduced considerably by oral administration of taurine (5 8). The normal deposits of taurine are small and the conservation of the taurine conjugated bile acids in the organism depends chiefly upon the active reabsorptive mechanism in the terminal ileum. As regards the glycine conjugates a passive nonionic diffusion along the entire small bowel is possible (pK_a for glycine conjugates is about 4.5), whereas this is not the case for the taurine conjugates to any noteworthy degree (pK_a for taurine conjugates is about 1.5)

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EXOCRINE PANCREATIC FUNCTION IN PORPHYRIA CUTANEA TARDA

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Abstract. Pancreatic exocrine function has been studied in 18 patients with porphyria cutanea tarda by determination of trypsin concentration in duodenal aspirate after standardized test meal. The results were compared with those obtained in group of 22 healthy subjects and in group of 18 chronic alcoholics without a history of pancreatic disease. There were no statistically significant differences between the means of the groups studied. Furthermore, no patient with porphyria cutanea tarda had a value below the range of the group of healthy non-alcoholic controls. In the group of chronic alcoholics one had value below the normal range.

Porphyria cutanea tarda symptomatica (PCT) is clinically characterized by skin fragility and blisters on skin areas exposed to the sun. Hypertrichosis and hyperpigmentation are common. The disease generally manifests itself in middle or late life, and males are much more commonly affected than females.

Biochemically there is gross uroporphyrinuria, while urinary excretion of coproporphyrin usually is slightly increased. In the typical case the excretion of porphyrin precursors (delta-aminolevulinic acid and porphobilinogen) is normal. Laboratory signs of liver disease are common and liver biopsy often reveals steatosis and fibrosis while, at least in Sweden, advanced cirrhosis of the liver is uncommon (15, 13). In PCT a slight or moderate iron overload is common (15). Pancreatic disease as a cause of increased iron stores in PCT has been suggested (10) and a decreased exocrine pancreatic function was said to be common in PCT but no data were given (20). Decreased exocrine pancreatic function in PCT was thought to be caused by abuse of alcohol, to which many patients with this disease are subject (10).

The main aim of the present investigation was to study whether the exocrine pancreatic function is decreased in PCT. One control group, comprising non-porphyric alcoholic without a history of pancreatic disease and group of non alcoholic healthy subjects are included in the study.

MATERIAL

The *porphyric series* comprised 18 patients (16 men and 2 women) with mean age of 59 years (range 45 to 76 years). Twelve had typical clinical and biochemical signs of manifest PCT at the time of the study. Six had been treated by phlebotomy as described by Lundvall and Weinfeld (15) and had clinically latent disease. These had earlier had typical clinical and biochemical signs of PCT. Liver biopsy performed in 14 patients showed varying degrees of steatosis in ten, and slight to moderate periportal fibrosis in eight. A marked periportal fibrosis was present in one. *Distortion of liver architecture as* in cirrhosis of the liver was not present in any patient. Abuse of alcohol of degree comparable with that of the alcoholic control group was factor in seven patients. Most of the others also had a rather large alcohol consumption.

The *alcoholic series* included 18 men with mean age of 47 years (range 38 to 55 years) admitted to mental hospital (Clinic II, Lillhagen Hospital) because of chronic and acute alcoholism. All these had well-documented record of abuse of alcohol for at least 10 years, many for 20 to 30 years. Alcoholics with history of pancreatitis (acute or chronic), with clinical signs of cirrhosis of the liver or who had undergone gastric resection, were not included. Liver biopsy was not performed in this group.

The *group of normals* included 22 healthy subjects (17 men and 5 women) with mean age of 35 years (range 18 to 70 years). In this group none had history of gastrointestinal disease, and there was no abuse of alcohol.

Five patients with clinical signs of pancreatic insufficiency were also included in the study. They all had gross steatorrhea.

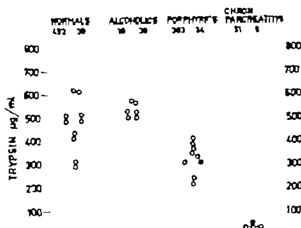


Fig 1 Trypsin activity in duodenal aspirate after a standardized test meal. Dotted lines, mean values.

METHODS

The pancreatic exocrine function was tested largely according to Lundh (12, 14) According to this method trypsin activity is determined in duodenal content collected after a standardized test meal. The intubation technique, the test meal and the principle of sampling were the same as described by Lundh (12). After the test meal, intestinal contents were collected (in bottles standing in crushed ice) for two hours at 10-min intervals during the first hour and at 30-min intervals during the second hour. One ml from each sample period was taken to a pool and the trypsin activity was determined on this pool. One ml of the pooled intestinal juice was pipetted into each of two centrifuge tubes with 9 ml absolute alcohol. Then the tubes were placed in crushed ice for half an hour and centrifuged The supernatant was decanted and the precipitate was redissolved in 9.5 ml of 0.9% NaCl. The trypsin activity in the solution was then determined with benzoyl DL-arginine *p*-nitroanilide hydrochloride (DL-BAPA) as substrate according to the method of Erlanger et al. (4) Substrate stock solution was prepared as follows. 43.5 mg DL-BAPA was dissolved in 1 ml dimethylsulfoxide and the solution was brought to 100 ml with 0.05 M Tris buffer pH 8.2, containing 0.02 M $CaCl_2$. Stock solution was prepared fresh at each analysis and placed in a thermostatically controlled bath at 37° Five ml substrate stock solution and 0.9 ml water were pipetted into 10 ml tubes placed into the bath (37°) and allowed to equilibrate for 20 min. At zero time 0.1 ml of the redissolved precipitate was pipetted into the test tube and the reaction was allowed to run for 600 sec. Then 1.0 ml of 30% acetic acid was added to terminate the reaction. The same amount was added to a blank tube. The extinction was read in a Beckman B spectrophotometer at 410 mμ and the extinction of the blank was subtracted. All determinations were made in duplicate. The enzymatic activity is given in μg/ml referring to a standard reference curve prepared on crystalline trypsin (Trypure, Novo, Copenhagen, Denmark).

RESULTS

The distribution of the trypsin values provided a satisfactory approximation to a normal distribution and differences between the means were tested with the conventional Student's *t*-test. The distribution of values were also tested according to a non-parametrical method viz. the Wilcoxon test (2).

The mean trypsin concentration of the 22 *normals* was 452 ± 39 (standard error of mean) μg/ml, and the range was 150 to 880 μg/ml. The mean value of 18 *PCT patients* was 363 ± 34 μg/ml and the range 175 to 775 (Fig 1). Thus no patient had a value below the range of the normals. The mean value in PCT was lower than normal, but the difference was not statistically significant ($t = 1.7$ D.F 38 $p > 0.10$). The mean value of PCT patients with latent disease (313 ± 43) was not higher than that of the others. The mean value of the 18 *alcoholics* was 410 ± 38 μg/ml (range 105 to 655 μg/ml). This mean value was not statistically different from that of the normals ($t = 0.7$ D.F 38 $p > 0.10$) or of the PCT patients ($t = 0.9$ D.F 34 $p > 0.10$). In the five patients with clinically manifest pancreatic insufficiency the trypsin activity varied from 0 to 50 μg/ml.

DISCUSSION

The value of determining pancreatic enzyme activity in duodenal aspirate after a standardized test meal in the evaluation of pancreatic exocrine function has been confirmed by many investigators (8, 12, 13 25 26). In this study patients with overt pancreatic insufficiency had very low enzyme concentrations, and there was no overlap between the values of the group of normals and those of patients with overt pancreatic insufficiency

It is well documented that there is an association between abuse of alcohol and pancreatic disease. Ingestion of alcohol may provoke acute pancreatic injury (1 3 11 17 24), and chronic alcoholism is common in patients with chronic pancreatitis (7 17 19). In autopsy materials structural pancreatic changes were reported to occur in a high frequency of chronic alcoholics (21 24). In cirrhosis of the liver irrespective of etiology a disturbed exocrine pancreatic function has also

been reported (5 6, 9) Hence a decreased exocrine pancreatic function might be expected to occur in porphyria cutanea tarda because abuse of alcohol and liver disease is common in this porphyria. However the present study does not indicate that decreased exocrine pancreatic function is common in chronic alcoholics without a history of pancreatitis. Only one out of 18 had a value below the lower range of [illegible]2 normals. In the present PCT patients there was no history of steatorrhea or pancreatitis, nor in 20 other patients with PCT known to the authors. The trypsin values were within the range of normals in all of the present 18 cases, and the mean value was not significantly lower than that of the normals. Thus the present study did not indicate that exocrine pancreatic function is decreased in porphyria cutanea tarda.

In a report by Saunders (20) from Cape Town, South Africa, it was stated that decreased pancreatic function was common in patients with porphyria cutanea tarda, though no data were given. In another report from the same center (University of Cape Town) cirrhosis of the liver was found in biopsy specimens of 11 out of 4[illegible] patients with porphyria cutanea tarda (22) A decreased concentration of exocrine pancreatic enzymes has been reported in cirrhosis of the liver (5 6, 9) and was thought to be caused by an increased bile secretion (5 6). Saunders statement may be founded on studies performed on porphyrics with more advanced liver disease than in the present series. In this country overt cirrhosis is not common in porphyria cutanea tarda (15 23) and was not found in any of the 14 PCT patients of this study examined by liver biopsy

There is some evidence indicating a reduced pancreatic exocrine function with advanced age (16), although this could not be confirmed by Ohlsén (18). The mean age of the group of normals (35 years) was significantly lower than that of the porphyric group (59 years). This difference in age distribution does not influence the results, as a normal mean value might have been slightly lower if older subjects had been studied.

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CLINICAL, METABOLIC AND CARDIOVASCULAR EFFECTS OF DIFFERENT PROSTAGLANDINS IN MAN

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Abstract. Prostaglandin A_1 (PGA_1), PGE_2, $PGF_{1\alpha}$, $PGF_{1\beta}$, and $PGF_{2\alpha}$ have been infused intravenously into nine healthy male subjects. PGA_1 was given to three subjects in doses from 0.056 to 0.56 μg/kg/min. In one of these subjects a slight flush was seen. The heart rate tended to increase without significant change in blood pressure. The levels of free fatty acids (FFA) in plasma increased slightly. PGE_2 was given to two subjects in doses from 0.056 to 0.56 μg/kg/min. A flush was seen in all four subjects given PGE_2, and the other effects were also similar to those previously seen with PGE_1. Thus an increase in heart rate and of plasma FFA levels occurred. With the prostaglandins of the F-series in doses up to 0.32 or 0.56 μg/kg/min, no clinical effects were noted. There were no significant changes in heart rate, blood pressure or plasma FFA levels.

The depressor and smooth muscle stimulating activity of the factor prostaglandin was first demonstrated by Goldblatt (19 20) and von Euler (16, 17 18). The chemical work on the prostaglandins was continued by Bergström (1 2, 3 4) during the last decade, and he showed that the factor prostaglandin consists of a family of related compounds occurring in various tissues of animals and man. From animal studies it is also clear that the different prostaglandins have various biological effects (9 16). However the physiological roles of all the various prostaglandins are not known as yet.

The first study of the effects of prostaglandins in man was done in 1959 by Bergström et al. (10) who infused prostaglandin E (PGE). After Bergström and Sjövall (11 12) in 1960 had isolated PGE_1 and $PGF_{1\alpha}$ from the prostate glands of sheep, several of the prostaglandins have become available as crystalline products, and it has been possible to study their various biological effects.

During recent years the clinical cardiovascular and metabolic effects of PGE_1 when infused intravenously into man have been studied (5 6 13 14). Some of the reported effects are cutaneous flush, headache, abdominal pains, increased heart rate and cardiac output and an increased mobilization of plasma free fatty acids (FFA).

In the present investigations we have studied two other prostaglandins from the E-series in man, namely PGE_1–217 (PGA) and PGE_2. The prostaglandins of the F-series show effects which are different from those of the E-series when studied in various animal species. The effects of the prostaglandins of the F-series have not been studied in man previously We here report the results from infusing $PGF_{1\alpha}$, $PGF_{1\beta}$ and $PGF_{2\alpha}$ in man. Clinical effects were registered, the blood pressure and heart rate recorded and the concentration of blood glucose and free fatty acids in blood plasma were followed. The technique was similar to that used in our previous studies with PGE_1 (13 14).

MATERIAL AND METHODS

Nine male volunteers, between 21 and 24 years of age, were studied. Four of them were studied twice on different occasions They were healthy as judged from routine clinical and laboratory investigations. Exercise tests did not show any abnormalities with respect to working capacity (23) or BCG The heart volume and the total amount of hemoglobin were also determined in some of the subjects, and the values were within normal limits.

The subjects reported at the laboratory at 8 a.m. after fasting at least 12 hours over night. One catheter of teflon was placed percutaneously into the brachial artery after local anesthesia with Carbocain ® (Bofors, Sweden).

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Table I. *Clinical effects of various prostaglandins, infused i.v. at increasing doses for 30 min periods as indicated in Figs. 1–5*

0 = no symptoms. A = abdominal pains. D = dyspnea. F = flush. H = headache. P = pallor
( ) = symptoms of moderate intensity

Subject	Prostaglandin infused (μg) /min/kg 0.056	0.10	0.18	0.3	0.56	After
PGA						
B. E.	F	(F)	(F)	0	—	0
A. V.	—	0	0	0	0	0
A. B.	—	0	0	0	0	0
PGE_2						
An. L.	0	(H)	(F)H	F H	—	H
A. M.	0	0	A, F →	P A, H, P	—	A, H
A. J.	—	0	0	F	F	0
J. T.	—	0	D F	D F	F	0
$PGF_{1\alpha}$						
Al. L.	0	0	0	0	—	0
U. E.	0	0	0	0	—	0
$PGF_{1\beta}$						
Al. L.	—	0	0	0	0	0
K. E.		0	0	0	0	0
$PGF_{2\alpha}$						
B. E.	0	0	0	0	—	0
A. B.	0	0	0	0	—	0

Another catheter was introduced into a vein of the opposite arm for infusion of the prostaglandins. With the catheters in place the subjects rested comfortably in supine position throughout the study.

The arterial catheter was used for blood sampling and also for blood pressure monitoring with an Elema-Schönander pressure transducer (EMT 490 A). The ECG was followed continuously during the study and the heart rate was calculated over several respiratory cycles from the ECG.

Prostaglandin E_1 217 (PGA_1), PGE_2, $PGF_{1\alpha}$, $PGF_{1\beta}$ and $PGF_{2\alpha}$ were obtained from S. Bergström, M.D. (Karolinska Institutet, Stockholm, Sweden) as crystalline preparations.

The different prostaglandins were dissolved in saline and sterilized by ultrafiltration. The sterile solutions containing 50 μg/ml were dispensed in 5 ml portions and stored at −15°C. The solutions were diluted in saline, 5 times volume, immediately before infusion.

Analysis

The arterial blood was withdrawn into heparinized syringes. Aliquots of blood were precipitated for determination of glucose, and the remainder was promptly centrifuged to separate cells from plasma. The plasma was immediately processed, and FFA were determined according to Dole (15) with the modification described by Trout et al. (14). Blood glucose was determined by the enzymatic method of Marks (22).

RESULTS

Clinical effects

The clinical effects are summarized in Table I. *PGA* produced a slight flush in the face and in the upper part of the body only in one of three subjects. Not even the highest dose, 0.56 μg/kg/min, caused any flush in the two other subjects. There were no other subjective or objective symptoms. PGE_2 produced a flush in the face in all four subjects studied. Two of the subjects complained of headache, and one of them also had abdominal cramps. One of the subjects complained of dyspnea. Generally these symptoms were of moderate intensity and it was not necessary to stop the infusions, not even when the highest dose 0.56 μg/kg/min was given. With the remaining three prostaglandins studied, $PGF_{1\alpha}$, $PGF_{1\beta}$ and $PGF_{2\alpha}$, there were no subjective or objective symptoms.

Blood pressure and heart rate

PGA_1 (Fig. 1). The mean blood pressure was almost unchanged in the three subjects throughout the study. The heart rate increased slightly during the infusion of PGA_1. However, after the infusion, the heart rate returned towards the initial frequency only in one of the subjects and remained increased in the remaining two. The mean levels of heart rate and blood pressure before and at the end of the infusion of 0.32 μg/kg/min of PGA_1 were 61 and 75 beats/min (range 60–62 and 69–82) and 84 and 79 mm Hg (range 82–86 and 78–82), respectively.

The corresponding figures with PGE_1 given to four subjects in a previous study (18) were, for heart rate, 65 and 96 beats/min (range 57–75 and 86–102) and, for blood pressure, 89 and 77 mm Hg (range 84–102 and 66–96).

PGE_2 (Fig. 2). The mean blood pressure did not change significantly during the infusion of PGE_2, but the heart rate increased in all subjects. The mean levels of heart rate and blood pressure before and at the end of the infusion of 0.32 μg/kg/min of PGE_2 were 60 and 77 beats/min (range 53–72 and 69–86) and 81 and 85 mm Hg (range 77–84 and 80–92), respectively. After the infusion of PGE_2 the heart rate decreased in all subjects toward a frequency similar to that before infusion.

$PGF_{1\alpha}$ (Fig. 3), $PGF_{1\beta}$ (Fig. 4) and $PGF_{2\alpha}$

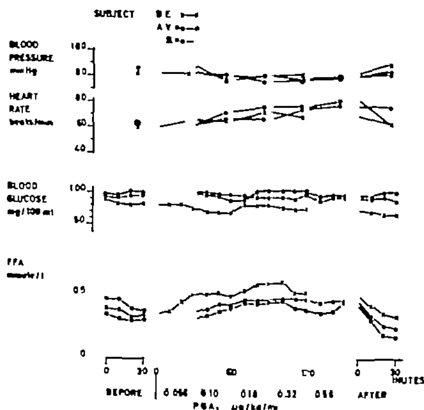


Fig. 1. Effect of PGA₁ on blood pressure, heart rate, blood glucose and free fatty acids (FFA) in plasma of three healthy subjects. PGA₁ given at constant rate in doses successively increased as indicated in the figure. The dose varied from 0.056 to 0.56 µg kg min.

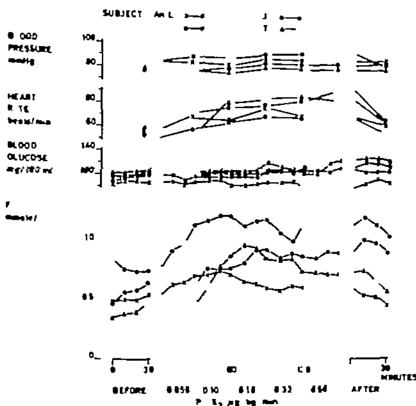


Fig. 2. Effect of PGE₁ on blood pressure, heart rate, blood glucose and free fatty acids (FFA) in plasma of four healthy subjects. PGE₁ given at constant rate in doses successively increased as indicated in the figure. The dose varied from 0.056 to 0.56 µg/kg/min.

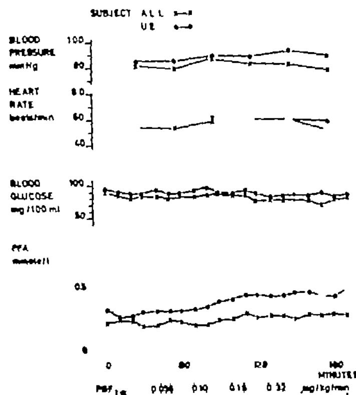


Fig. 3. Effect of $PGF_{1\alpha}$ on blood pressure, heart rate, blood glucose and free fatty acids (FFA) in plasma of two healthy subjects. $PGF_{1\alpha}$ given i.v. at constant rate in doses successively increased as indicated in the figure. The dose varied from 0.056 to 0.32 µg/kg/min.

(Fig. 5). Except for one of the subjects Al. L., receiving $PGF_{1\beta}$, there were almost no changes in blood pressure or heart rate in the subjects given the prostaglandins of the F-type. In subject Al. L. the heart rate increased during administration of the highest doses of $PGF_{1\beta}$, 0.32 and 0.58 µg/kg/min and decreased after the infusion (Fig. 4).

Metabolic effects

PGA_1 (Fig. 1). During infusion of the two lowest doses, 0.056 and 0.10 µg/kg/min, the arterial plasma FFA levels tended to increase slightly in all subjects. When the next two doses, 0.18 and 0.32 µg/kg/min, were given there were no consistent changes of FFA. After the infusion of PGA_1 there was a drop in the FFA concentration in the three subjects studied. No significant changes in the blood glucose concentration were seen.

PGE_2 (Fig. 2). The infusion of PGE_2 was started with 0.056 µg/kg/min in two of the subjects, and with 0.10 in the remaining two. There was an initial increase in FFA concentration in all subjects, the maximal rise varying from 0.21 to 0.49 mmole/l (mean 0.31) during the first 50 min. After that there were no consistent changes. During the 30 min-period after infusion the FFA concentration decreased in all four subjects. There were no consistent changes in the blood glucose concentrations.

$PGF_{1\alpha}$ (Fig. 3). There were no changes in the concentrations of FFA and blood glucose, either during or after the administration of $PGF_{1\alpha}$.

$PGF_{1\beta}$ (Fig. 4). In one of the subjects the FFA concentration increased rapidly already before administration of $PGF_{1\beta}$. During infusion of the prostaglandin into the subject there were no consistent changes in the FFA level, and it remained unchanged after the infusion. In the other subject the FFA level was almost unchanged throughout the study. The blood glucose levels were unchanged in the two subjects.

$PGF_{2\alpha}$ (Fig. 5). There were no consistent changes in the concentration of FFA and blood glucose during infusion of $PGF_{2\alpha}$, and the levels were almost unchanged after the infusion.

DISCUSSION

The effects of prostaglandin A_1 have previously been compared (7) with the effects of PGE_1 on the blood pressure and heart rate in the dog. It

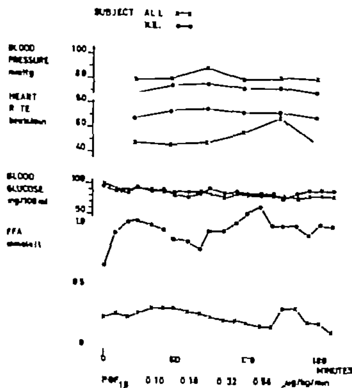


Fig. 4. Effect of PGF$_{1\beta}$ on blood pressure, heart rate, blood glucose and free fatty acids (FFA) in plasma of two healthy subjects. PGF$_{1\beta}$ given at constant rate in doses successively increased as indicated in the figure. The dose varied from 0.10 to 0.56 g/kg/min.

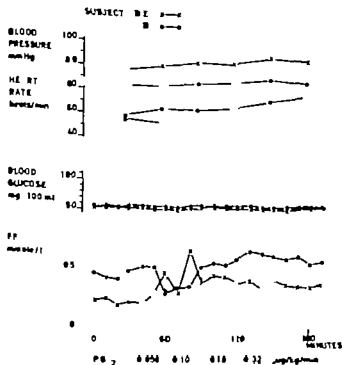


Fig. 5. Effect of PGF$_{2\alpha}$ on blood pressure, heart rate, blood glucose and free fatty acids (FFA) in plasma of two healthy subjects. PGF$_{2\alpha}$ given at constant rate in doses successively increased as indicated in the figure. The dose varied from 0.056 to 0.32 µg/kg/min.

was found that PGA_1 was at least as effective as PGE_1 in lowering the blood pressure and increasing the heart rate. In contrast to the results in dogs, the present studies indicate that PGA_1 appears to be less effective than PGE_1 in influencing the cardiovascular system in man. A slight flush was here seen with PGA_1 only in one of the three subjects. In contrast PGE_1 always causes a pronounced flush when given as an intravenous infusion in the same doses as PGA_1 was given in the present study (13) When PGE_1 is infused into man, symptoms like headache and abdominal pains are also induced. Because of these effects, the maximal tolerable dose for PGE_1 infused intravenously varies between 0 10 and 0.3 $\mu g/kg/min$. In this study one subject received 0.32 and the remaining two 0.56 $\mu g/kg/min$ of PGA_1 as a maximal dose without any disturbing symptoms.

PGE_1 in vitro has an inhibitory effect on lipolysis in adipose tissue from man (9). In vivo, however PGE_1 increases the mobilization of free fatty acids when infused intravenously (13). From these findings and from studies on dogs with PGE_1 (8 9) it appears possible that the effect of PGE_1 on FFA mobilization in vivo may be due to at ... two mechanisms, one with direct inhibition ... another with indirect stimulation of FFA ...lization.

PGA_1 has no inhibitory effect on lipolysis in adipose tissue in vitro (7). One might therefore possibly expect a more pronounced increase of FFA concentration in vivo with PGA than with PGE if PGA_1 had a stimulatory effect on the hypothetical indirect mechanism responsible for the PGE_1-induced rise in FFA concentration. During the infusion of PGA into man the FFA levels here tended to increase initially and decrease after the infusion, which is the same type of response as seen with infusion of PGE_1 However the changes in FFA induced by PGA appeared to be much less pronounced than the changes seen previously with PGE_1 (13).

With PGE_2, similar clinical and metabolic effects were seen as with PGE_1 (13 14). Although higher doses were given of PGE_2, the effects were not so pronounced as with PGE_1 and it was in no case necessary to interrupt the infusion. This finding suggests a quantitative but not qualitative difference between the actions of PGE_1 and PGE_2 in man. With the prostaglandins of the F-series here studied, $PGF_{1\alpha}$, $PGF_{1\beta}$ and $PGF_{2\alpha}$ no clinical, cardiovascular or metabolic effects were seen which suggest that their biological function and/or metabolism must be different from that of PGE_1 The results with $PGF_{2\alpha}$ are in agreement with those recently reported by Karim et al. (11)

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